UNIVERSIDAD AUTÓNOMA DE MADRID

FACULTAD DE MEDICINA

DEPARTAMENTO DE MEDICINA PREVENTIVA Y SALUD PÚBLICA

PhD Thesis

THE ROLE OF GENOMIC DNA METHYLATION IN THE RISK AND PROGRESSION OF UROTHELIAL CARCINOMA OF THE BLADDER

Salman Muhammad Tajuddin

Spanish National Cancer Research Centre (CNIO)

Madrid, 2013

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La Dra. Núria Malats Riera, Jefa del Grupo de Epidemiología Genética y Molecular, y el Dr. André Filipe Santos Amaral, del Centro Nacional de Investigaciones Oncológicas (CNIO), como Directores.

CERTIFICAN:

Que Don Salman Muhammad Tajuddin, Licenciado en Medicina por la Jimma University y con el grado de Master en Salud Publica por la Hebrew University of Jerusalem, ha realizado la presente Tesis Doctoral **"The role of genomic DNA methylation in the risk and progression of urothelial carcinoma of the bladder"** y que a nuestro juicio reúne plenamente todos los requisitos necesarios para optar al **Grado de Doctor**, a cuyos efectos será presentada en la Universidad Autónoma de Madrid, autorizando su presentación ante el tribunal Calificador.

Y para que así conste se extiende el presente certificado,

Madrid, 5 de abril de 2013.

Vº Bº de la Directora de la Tesis:

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ACKNOWLEDGMENTS

I would like extend my deepest appreciations to my supervisors Dr. Núria Malats and Dr. André F. S. Amaral. Thank you so much for giving me the opportunity to work in this exciting project, for your valuable support, constructive criticisms and guidance throughout the research.

I want also thank my thesis committee members Dr. Mercedes Robledo, Dr Francisco X. Real, and Dr. Mario F. Fraga for your advice. This thesis has benefited from the many discussions I had with you during my research. I also want to thank Dr. Mario Fraga and Dr. Agustin F. Fernandez for DNA methylation analyses and for their valuable contributions.

I want to extend my gratitude to the past and present members of the Genetic and Molecular Epidemiology group for creating an exciting learning and working environment. Thanks to the Spanish Bladder Cancer Study/EPICURO study participants and investigators, without whom this project would not have been possible.

Thank you to Dr. Fernando Rodríguez Artalejo and Milagros Santos at the Department of Preventive Medicine and Public Health of Universidad Aurónoma de Madrid for their guidance and assistance.

I would like to thank the Association for International Cancer Research (AICR) for their generous financial support for my PhD fellowship. I also want to acknowledge the academic and technical support of the Spanish National Cancer Research Centre (CNIO).

Last but not least, I want to extend my deepest gratitude to my dear parents, sisters and brothers for your encouragement and for everything you have done for me. This thesis is for you all.

FUNDING

The PhD thesis was partially supported by the Association for International Cancer Research (AICR, #09-0780, including a PhD scholarship awarded to SMT); Fondo de Investigaciones Sanitarias, Instituto de Salud Carlos III, MINECO, Spain (00/0745, PI051436, PI061614, PI09-02102, and G03/174); Red Temática de Investigación Cooperativa en Cáncer-RD06/0020-RTICC; USA-NIH-RO1-CA089715; a postdoctoral fellowship awarded to AFSA from the Fundación Científica de la AECC; Fundació Marató TV3.

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LIST OF ABBREVIATIONS

BCG	Bacille Calmette-Guérin
BCSM	Bladder cancer-specific mortality
CIS	Carcinoma in situ
DUX4	Double homeobox 4
GSTM1	Glutathione S-transferase mu 1
GSTT1	Glutathione S-transferase theta 1
HR	Hazard ratio
ISUP	International Society of Urological Pathology
LINE-1	Long interspersed nucleotide element-1
LRT	Likelihood ratio test
LTR	Long terminal repeat
MIBC	Muscle-invasive bladder cancer
NAT2	*N*-acetyltransferase 2
NMIBC	Non-muscle-invasive bladder cancer
PEMT	Phosphatidylethanolamine *N*-methyltransferase
OM	Overall mortality
OR	Odds ratio
PITX1	Paired-like homeodomain 1
PUNLMP	Papillary urothelial neoplasm of low malignant potential
SAM	*S*-adenosylmethionine
SBCS/EPICURO	Spanish Bladder Cancer Study/Epidemiology of cancer of the urothelium
SNP	Single nucleotide polymorphisms
TURBT	Transurethral resection of bladder tumor
UCB	Urothelial carcinoma of the bladder

UTR	Untranslated region
WHO	World Health Organization

ABSTRACT

Urothelial carcinoma of the bladder (UCB) is one of the most common cancer types in the developed world, especially in men. Established risk factors for UCB are tobacco smoking, occupational and exposure to aromatic amines. Recently, 12 genetic variants have been reported to be associated with UCB, among them those in *NAT2* and *GSTM1*; these genes modify the risk conferred by smoking. DNA methylation plays an important role in cellular differentiation and growth, and altered methylation levels in cancer tissues are a hallmark of the disease. Both regional and global DNA methylation changes have been observed in UCB tissue. DNA methylation levels may be influenced by genetic and environmental factors but are poorly characterized. Thus, the objectives of this thesis are to identify predictors of genomic DNA methylation (measured at LINE-1 and D4Z4 repeat sequences in granulocyte DNA); to investigate the association between genomic DNA methylation and the risk and progression of UCB, and; to identify modifiers of these associations.

In this thesis, methylation levels in bisulfite-treated granulocyte DNA were determined by pyrosequencing at LINE-1 (952 cases and 892 controls) and D4Z4 (707 cases and 718 controls) repeats from subjects of the Spanish Bladder Cancer/EPICURO Study. Data on sociodemographic characteristics, smoking, trace elements, single nucleotide polymorphisms (SNPs) in 24 one-carbon metabolism pathway genes, clinicopathological, and follow-up data were included in the analyses. Multivariable robust linear regression, logistic regression and Cox proportional hazards regression were used to identify potential predictors of genomic DNA methylation, assess its association with risk, and determine its prognostic potential in UCB, respectively. Likelihood ratio test was used

to compare the models with and without the multiplicative interaction term and estimate the interaction p-value.

LINE-1 methylation levels were associated with gender, tobacco type, arsenic, iron, and nickel, and seven genetic variants in one-carbon metabolism pathway genes (*DNMT3A*-rs7581217, *AS3MT*-rs7085104, *MTHFS*-rs1380642, *SLC19A1*-rs914238, and rs9621049, rs9606756, rs4820887 in *TCN2*). D4Z4 methylation levels were associated with gender, tobacco type, and selenium. LINE-1 methylation showed a U-shaped association with risk of UCB. Both the lowest and highest tertiles were associated with increased risk of UCB when compared to the middle tertile. This association was modified by five SNPs in the *PEMT* gene. Individuals with D4Z4 methylation levels above or equal to the median had a slightly increased risk of UCB. D4Z4 hypermethylation was associated with significantly increased risk of low-grade non-muscle-invasive bladder cancer overexpressing FGFR3. The overall risk conferred by D4Z4 hypermethylation was significantly modified by iron, manganese, and zinc, and *FOLH1*-rs11040387. LINE-1 methylation and D4Z4 methylation levels were not associated with individual outcomes of UCB.

In summary, personal characteristics, environmental and common genetic polymorphisms in one-carbon metabolism pathway genes were associated with genomic DNA methylation. Genomic DNA methylation was associated with increased risk of UCB, and this risk was modified by trace elements and genetic polymorphisms. These results show the potential of genomic DNA methylation as a biomarker of susceptibility to UCB. Further studies in larger populations are required to confirm these findings.

RESUMEN

El carcinoma urotelial de vejiga (CUV) es uno de los tipos de cánceres más comunes en el mundo desarrollado, principalmente en hombres. Los factores de riesgo establecidos para el CUV son fumar, exposición ocupacional a las aminas aromáticas. Recientemente, se ha visto que 12 variantes genéticas están asociadas con el CUV, entre ellas las que se encuentran en *NAT2* y *GSTM1*; estos genes modifican el riesgo que confiere el tabaco. La metilación del ADN juega un importante papel en la diferenciación y crecimiento celular y la alteración de los niveles de metilación en los tejidos tumorales son marcadores de la enfermedad. Se han observado cambios en la metilación del ADN, tanto global como regional en tejido del CUV. Los niveles de metilación del ADN pueden verse influenciados por factores genéticos y ambientales, pero éstos están poco caracterizados por el momento. Por lo tanto, los objetivos de esta tesis son identificar los predictores de la metilación del ADN genómico (medida en las secuencias repetidas de LINE-1 y D4Z4 del ADN de granulocitos), para estudiar la asociación entre la metilación del ADN genómico y el riesgo y progresión del CUV, así como identificar modificadores de esta asociación.

Para realizar la tesis, los niveles de metilación en el ADN de granulocitos tratado con bisulfito fueron determinados por pirosecuenciación en LINE-1 (952 casos y 892 controles) y D4Z4 (707 casos y 718 controles) elementos repetitivos de sujetos del Estudio Español de Cáncer de Vejiga/EPICURO. El análisis se incluyeron datos sociodemográficos, consumo de tabaco, elementos traza, polimorfismos de un solo nucleótido (SNPs) en 24 genes de la via del metabolismo del monocarbono, clínico-patológicos y de seguimiento. Se realizó un análisis multivariante de regresión lineal robusta, regresión logística y regresión de Cox de riesgos proporcionales para identificar potenciales predictores de la metilación

del ADN, evaluar su asociación con el riesgo, y determinar su potencial pronóstico del CUV, respectivamente. Se utilizó el "*likelihood ratio test, LRT"* para comparar los modelos en presencia o ausencia del término de interacción multiplicativa y estimar el p-valor de la interacción.

Los niveles de metilación de LINE-1 se asociaron con género, tipo de tabaco, arsénico, hierro, y níquel, y siete variantes genéticas en genes de la via del metabolismo del monocarbono (*DNMT3A*-rs7581217, *AS3MT*-rs7085104, *MTHFS*-rs1380642, *SLC19A1*-rs914238, y rs9621049, rs9606756, rs4820887 en *TCN2*). Los niveles de metilación de D4Z4 se asociaron con género, tipo de tabaco y selenio. La metilación de LINE-1 mostró una asociación en forma de U con el riesgo del CUV. Tanto el tertil más bajo como el más alto se asociaron con un aumento del riesgo de CUV cuando se comparó con el tertil del medio. Esta asociación estaba modificada por cinco SNPs en el gen *PEMT*. Individuos con niveles de metilación igual o superiores a la media tenían un ligero aumento del riesgo de CUV. La hipermetilación de D4Z4 se asoció con un aumento significativo del riesgo de cáncer de vejiga no músculo invasivo de bajo grado con sobreexpresión de FGFR3. El riesgo global que supone la hipermetilación de D4Z4 estaba modificado de manera significativa por el hierro, manganeso, y zinc y *FOLH1*-rs11040387. La metilación de LINE-1 y los niveles de metilación de D4Z4 no estaban asociados con el pronóstico del CUV.

En resumen, características personales, ambientales y polimorfismos genéticos comunes en genes de la vía del metabolismo del monocarbono se asociaron con metilación del ADN genómico. La metilación del ADN genómico se asoció con un aumento del riesgo de CUV, y ese riesgo estaba modificado por elementos traza y polimorfismos genéticos.

Estos resultados muestran el potencial de la metilación del ADN genómico como un biomarcador de susceptibilidad al CUV. Se precisan estudios adicionales en poblaciones más amplias para confirmar estos hallazgos.

CHAPTER I. GENERAL INTRODUCTION

1. Epidemiology of urothelial carcinoma of the bladder

Bladder cancer was the ninth most frequent cancer type worldwide with an estimated 382,660 new cases and 150,282 deaths, for both sexes combined, in 2008. It ranks second among urogenital malignancies (Ferlay *et al.* 2010). Bladder cancer is histologically classified into major categories as urothelial (transitional cell) carcinoma, squamous cell carcinoma, adenocarcinoma, and others. Urothelial carcinoma of the bladder (UCB) is the most frequent type of bladder cancer, accounting for about 90% of all cases in the Western world, followed by squamous cell carcinoma and adenocarcinoma. It predominantly affects men, with the male to female ratio ranging from 3 to 5 (Parkin 2008). In Europe, the highest incidence rates are observed in Denmark, Spain and FYR Macedonia with age-standardized incidence rates (world) of 14.5, 14.1, and 14.1 per 100,000, respectively (Ferlay *et al.* 2010). In Spain, the past three decades have seen an increase in the incidence rate of bladder cancer while mortality rate decreased in both sexes (Izarzugaza *et al.* 2010). Yet Spanish men have one of the highest mortality rates in Europe (age-standardized mortality rate of 8.3 per 100,000) (Ferlay *et al.* 2010). In Spain, the male to female ratio for this cancer is the highest with 7:1(Izarzugaza *et al.* 2010).

At initial presentation, the majority of UCB patients (75-85%) are diagnosed with non-muscle-invasive bladder cancer (NMIBC), and the rest with muscle-invasive bladder cancer (MIBC) (Falke & Witjes 2011). Depending on their tendency to recur and progress to advanced disease, NMIBC is further divided into low- and high-risk subgroups. Low-risk NMIBC tumors have a higher tendency for recurrence, whereas high-risk NMIBC tumors are prone to progression to muscle-invasive disease and metastasis (Donat 2003). Low-risk NMIBC are comprised of TaG1 and TaG2 tumors, while high-risk NMIBC includes carcinoma in situ (CIS, Tis), TaG3 and T1 tumors. Tumors with stages T2, T3 and T4 constitute the MIBC group. The risk of recurrence in non-muscle invasive tumors is 15-70% while approximately 7-40% progress to muscle-invasive disease, although these rates may vary because of tumor heterogeneity (Kaufman *et al.* 2009, Prasad *et al.* 2011). On the other hand, muscle-invasive disease is a life-threatening condition with 25-80% of tumors eventually developing into metastatic disease and 5-year survival being between 27-67% (Prasad *et al.* 2011).

Current molecular evidence indicates that UCB generally develops along two distinct pathways with genetic alterations in known tumor suppressor genes and oncogenes. Non-invasive tumors are characterized by gain-of-function mutations in *FGFR3* (50-60% of tumors), *PIK3CA* and *HRAS*, whereas high grade invasive tumors are characterized by loss-of-function mutations in *TP53* and *RB* genes (Knowles 2008, Wu 2005, Falke & Witjes 2011). Apart from specific gene mutation, deletion and genomic instability have been identified that are thought to be early events in bladder tumor development. The most frequent genomic alteration is seen in chromosome 9 where more than 50% of tumors, regardless of tumor stage and grade, display loss-of-heterozygosity at chromosome 9 (Knowles 2008). Additional frequent cytogenetic alterations include deletion at chromosomes 8p, 10p, 10q, 11p and gain at 1q, 17q and 20q (Knowles 2008).

1.1. Risk factors for urothelial carcinoma of the bladder

Incidence rates of UCB peak at age of 75 years and above, and its development is multifactorial in origin, mainly related to exposure to environmental carcinogens (Ferlay *et al.* 2010). Established risk factors include tobacco smoking, occupational exposure to aromatic amines and exposure to arsenic in drinking water (Delclos & Lerner 2008, Murta-Nascimento *et al.* 2007, Burger *et al.* 2013, Volanis *et al.* 2010).

Tobacco smoking is the most important risk factor for UCB. Tobacco smoke contains a multitude of carcinogens such as aromatic amines (β-naphthylamine, nitrosamines) and oxygen free radicals. The risk estimates for current smokers is between three and four relative to never smokers (Freedman *et al.* 2011), with smoking accounting for approximately 50-65% of the cases in men and 20-52% in women. The relative risk for former smokers is close to two compared to never smokers (Murta-Nascimento *et al.* 2007, Freedman *et al.* 2011). With changes over the past decades in the prevalence of tobacco smoking in both men and women, a recent study showed the proportion of UCB cases attributable to tobacco smoking to be approximately 50% in both sexes (Freedman *et al.* 2011) suggesting factors other than smoking may explain the elevated male-to-female ratio of developing the disease. Additionally, the type of tobacco smoked has been shown to have a differential effect on the risk of UCB, during and after cessation of smoking. The risk of developing the disease for smokers of black tobacco has been shown to be higher than that of

smokers of blond tobacco. Moreover, it has been shown that for smokers of blond tobacco an inverse association between risk and time since quitting might exist (Samanic *et al.* 2006).

Another risk factor for UCB is occupational exposure to carcinogens such as polycyclic aromatic hydrocarbons and aromatic amines (4-aminobiphenyl, benzo[*a*]pyrene, benzidine, β-naphthylamine, *N*,*N*-bis(2-chloroethyl)-2-naphthylamine (chlornaphazine), 4,4´-methylene bis(2-chloroaniline) (MOCA), and ortho-toluidine) (Delclos & Lerner 2008). It is estimated that about 30% of UCB cases are attributable to occupational exposures (Delclos & Lerner 2008).

Arsenic is a known carcinogen to humans causing cancer at different sites, including urinary bladder when exposure to arsenic is through drinking water. Inorganic arsenic is metabolized through a series of methylation reactions to monomethylarsonic and dimethylarsinic acids, themselves carcinogens, before being excreted through urine (Murta-Nascimento *et al.* 2007, Straif *et al.* 2009). Other potential risk factors include reduced fluid intake, and exposure to trihalomethanes in water (Murta-Nascimento *et al.* 2007, Burger *et al.* 2013). Individuals with lower serum vitamin D levels were found to have significantly increased risk of aggressive types of UCB and of tumors that express low levels of FGFR3 (Amaral *et al.* 2012a).

Medical conditions such as diabetes mellitus, chronic cystitis, bladder calculi, and treatments such as use of phenacetin-containing analgesics, cyclophosphamide, chlornaphazine, pioglitazone and pelvic irradiation have been found to be associated with UCB development (Murta-Nascimento *et al.* 2007, Burger *et al.* 2013). On the other hand, use of aspirin is inconsistently associated with decreased risk of UCB (Bosetti *et al.* 2012).

So far, the protective effect of fruit and vegetable consumption against UCB has been inconsistent and a recent large prospective study has found no clear association (Murta-Nascimento *et al.* 2007, Burger *et al.* 2013). Selenium intake has been shown to be inversely associated with UCB risk (Amaral *et al.* 2010, Rayman 2012).

Family history of UCB in first-degree relatives is associated with a two-fold increase in risk of developing the disease (Burger *et al.* 2013). Germline variations in *NAT2* resulting in a slow-acetylator phenotype and loss of both copies of *GSTM1* gene are the two best established genetic risk factors for UCB. In addition, multiplicative interactions between these genetic variations and smoking have been shown (Rothman *et al.* 2010, Garcia-Closas *et al.* 2005). More

recently, a series of genome-wide association studies and fine mapping studies on UCB identified additional genetic susceptibility variants in the following genes: *APOBEC3A, CCNE1, CLPTM1L, MYC, NAT2, PSCA, SLC14A1, TACC3/FGFR3, TERT, TP63* and *UGT1A6* (Tang *et al.* 2012, Garcia-Closas *et al.* 2011, Rothman *et al.* 2010, Wu *et al.* 2009, Rafnar *et al.* 2009, Kiemeney *et al.* 2008). Further, risk-difference modifications between smoking status and susceptibility variants in *NAT2, MYC, PSCA, CBX6/APOBEC3A, UGT1A6*, and *GSTM1* copy number variation have been reported (Garcia-Closas *et al.* 2013).

1.2. Diagnosis, staging, grading and treatment of urothelial carcinoma of the bladder

UCB is a heterogeneous disease with diverse morphologic and clinical manifestations. The most salient presentation of UCB is painless, intermittent hematuria. More than three-quarters of patients present with gross hematuria. The rest present as nonspecific irritating voiding symptoms, including urgency, frequency of urination and dysuria; symptoms frequently misinterpreted as urinary tract infection but that may signify either trigone involvement with tumor or the presence of CIS (Lopez-Beltran 2008, Prasad *et al.* 2011). Initial evaluation and management of patients with suspected bladder cancer involves invasive procedures such as cystoscopic evaluation of the bladder, transurethral resection of visible tumor for staging and grading, and assessment of the appearance of the uninvolved bladder and prostatic urethra (Lopez-Beltran 2008). In addition, urine cytology has an important role in detecting high risk UCB (Prasad *et al.* 2011).

Staging and grading are the most important determinants of patient outcome and decision on treatment and follow-up regimens, therefore accurate staging and grading is critical (Prasad *et al.* 2011, Lopez-Beltran 2008). Staging is often challenging, and substantial interobserver and intraobserver variability has been observed. Similarly, grading and classification of UCB has been controversial, with the grading of papillary urothelial carcinomas being the most debated (Lopez-Beltran 2008). In a bid to improve the classification system and reflective of this lack of consensus, several grading schemes for bladder cancer have been developed. These are the World Health Organization (WHO) 1973 classification (grading as: papilloma, grades 1 to 3), the 1998 WHO/ISUP classification [papilloma, papillary urothelial neoplasm of low malignant potential (PUNLMP), low grade, high grade], the 1999 WHO classification (papilloma, PUNLMP, grades

1 to 3), and the 2004 WHO classification systems (papilloma, PUNLMP, low grade, high grade), which is identical to the 1998 system. Grading with both the WHO 1973 and 2004 classification systems is recommended, although the 2004 system has less interobserver variability than the former (Liedberg *et al.* 2012, Cheng *et al.* 2012). On the other hand, clinical and emerging data from whole-genome gene expression, chromosomal aberrations, and mutation analyses support the use of the 1999 WHO classification system, particularly the separation of grade 3 from grade 2 in high grade tumors (Liedberg *et al.* 2012). There are ongoing efforts to replace the existing bladder tumor grading and classification system. Recently, Cheng *et al.* proposed a new four tier numerical grading system (as papilloma, grades 1 to 4) that incorporates the strengths of both the 1973 and the 2004 grading systems and abandons the terminology "PUNLMP" (Cheng *et al.* 2012). Incorporation of molecular grading, based on biomarker expression profiles, in the grading process may provide highly objective assessment of the tumors' biologic potential and help differentiate patients at risk of adverse clinical outcome (Cheng *et al.* 2012).

Treatment of UCB is dictated by the tumor stage, grade, size and number of tumors detected as well as the patient's performance status. Although treatment is individualized and can differ from patient to patient, generally, CIS is treated with transurethral resection of bladder tumor (TURBT) and intravesical immunotherapy by bacille Calmette-Guérin (BCG), which is an attenuated strain of *Mycobacterium bovis* used for immunization against tuberculosis. Low grade tumors Ta and T1 are treated by TURBT and/or subsequent intravesical instillation of BCG or single dose immediate chemotherapy with one of mitomycin C, epirubicin, or doxorubicin. High grade T1 tumors are managed either by radical cystectomy or TURBT and intravesical BCG or chemotherapy. Because of the risk of recurrence and progression, follow-up by periodic cystoscopy and urinary cytology for secondary tumors is required for both low- and high-risk NMIBC patients (Babjuk *et al.* 2011). MIBC can be treated by one of the following treatment options: radical cystectomy only; neoadjuvant chemotherapy and radical cystectomy; radical cystectomy followed by adjuvant chemotherapy; neoadjuvant chemotherapy, chemotherapy and irradiation when bladder preservation is indicated. Metastatic disease is managed by cisplatin-containing combined systemic chemotherapy, followed by selective surgery or irradiation (Stenzl *et al.* 2011).

1.3. Prognostic factors for urothelial carcinoma of the bladder

Both NMIBC and MIBC show different clinical outcomes. Low-risk NMIBCs are prone to recurrence, while high-risk NMIBCs are usually associated with increased rate of progression (Hernandez *et al.* 2006). Unlike other cancer types, mortality rate from UCB has changed very little over the past 20 years (Malmstrom 2011). Besides, the increased rate of recurrence and progression associated with NMIBC requires repeated cystoscopy, and long-term follow-ups for continued treatment, surveillance and monitoring. This creates a burden on the health care system and, in fact, UCB is the most expensive cancer to treat per patient (Botteman *et al.* 2003). Although there is no available data, indirect costs in the form of potential loss of work and productivity, decreased quality of life are also associated with the disease. Currently, established prognostic factors in NMIBC include grade, stage, presence of associated CIS (dysplasia), multifocality, tumor size, frequency of recurrence, and depth of lamina propria invasion. Prognostic factors for MIBC are stage, presence of lymphovascular invasion and presence of divergent histology (Netto 2012). Although these conventional prognostic factors are widely used, they fail to clearly evaluate each individual tumor's malignant potential (Lopez-Beltran 2008). These shortcomings indicate a need for the development of additional molecular prognostic markers to improve the accuracy of the existing clinicopathology-based prognostic scoring system in identifying patients who might benefit from aggressive and more intensive treatment (Netto 2012).

Apart from the abovementioned clinicopathological parameters, a growing number of potential molecular prognostic markers are being identified for both NMIBC and MIBC. Emerging molecular prognostic markers in NMIBC include *FGFR3* mutation and overexpression, *HRAS* alteration, proliferation indices, loss of E-cadherin, overexpression of angiogenesis markers, and markers for MIBC include *TP53* inactivation, alteration of *RB*, p16 expression, loss of E-cadherin, overexpression of angiogenesis markers (Netto 2012). Mutations in *FGFR3* are associated with increased risk of recurrence and decreased rate of progression (Hernandez *et al.* 2006), while *HRAS* is overexpressed in tumors with low progression potential. On the other hand, tumors with altered *TP53* exhibit the worst prognosis (Mitra & Cote 2009). In addition to somatic prognostic markers, germline polymorphisms have been identified through candidate-gene and genome-wide prognostic studies. Polymorphisms in genes involved in critical cellular processes such as cell cycle (*TP53*, *MDM2*), DNA repair, inflammation and oxidative

stress have been associated with various end points of the disease (Chang *et al.* 2012). A recent multi-stage genome-wide prognostic study has identified common germline polymorphisms associated with recurrence, progression and relapse of NMIBC (Malats *et al.* 2013).

2. Epigenetics

Epigenetics refers to the study of "a stably heritable phenotype resulting from changes in a chromosome without alterations in the DNA sequence" and passed on through cell division - either mitosis or meiosis (Berger *et al.* 2009). The epigenome refers to the epigenetic overall state of the cell. Epigenetic changes include DNA modifications [5-methylcytosine, 5-hydroxymethylcytosine, 5-formylcytosine, 5-carboxylcytosine (Ito *et al.* 2011, Bhutani *et al.* 2011)], post-translational modifications of histone proteins [methylation, acetylation, and several other modifications of the N-terminal of amino acids of histones (Tan *et al.* 2011)], chromatin remodeling (nucleosome positioning), and non-coding RNAs. The combination of these epigenetic modifications impact transcription factors or other chromatin binding proteins, thereby regulating gene expression and maintaining genomic stability (Berger *et al.* 2009).

2.1. DNA methylation

DNA methylation is the most studied and fundamental epigenetic modification. It corresponds to the covalent addition of a methyl group to DNA at the 5´position of the cytosine pyrimidine ring. DNA methylation is dynamic over time and is established and continually maintained by DNA methyltransferases (DNMT1, DNMT3A and DNMT3B). DNMT3A and DNMT3B are primarily *de novo* methyltransferases, while DNMT1 maintains the methylation marks after DNA replication in cooperation with the *de novo* methyltransferases (Fernandez *et al.* 2012, Jones & Liang 2009, Bird 2002). Cytosine methylation predominantly occurs in the context of CpG dinucleotides that are asymmetrically distributed over the human genome. Although rare, non-CpG methylation has recently been described in humans at CHG and CHH sites where H can be any of the three other nucleobases (A, C, T) (Portela & Esteller 2010). CpG dinucleotides constitute approximately 1% of the genome and are mostly found in CpG islands -located close to most gene promoters, CpG islands are genomic regions with more than 200 bases with a G+C content of at least 50% and a ratio of observed to statistically expected CpG frequencies of at least 0.6, CpG island shores -located up to 2 kb upstream of the CpG island, gene bodies, repetitive genomic sequences -long interspersed nucleotide element-1 (LINE-1), *Alu* and sort interspersed nucleotide element (SINE), and tandem repeats -such as D4Z4 (subtelomeric

regions), *NBL2*, pericentromeric satellite 2 and 3 repeats, alpha satellite sequences (*Satα*) (Portela & Esteller 2010, Sandoval *et al.* 2011).

The function of DNA methylation is dependent on the context of the genome, and when disrupted it leads to altered gene expression and phenotypic consequences. DNA methylation plays a fundamental role in long-term silencing of retrotransposons, germline and tissue-specific genes, imprinting, and X-chromosome inactivation thereby ensuring normal cellular differentiation, growth and development (Jones 2012). CpG islands at transcription start sites are usually unmethylated in normal cells and methylation in these regions is associated with repression of gene expression (Portela & Esteller 2010, Jones 2012). This repression of gene expression is achieved by mechanisms such as recruitment of methyl-CpG-binding domain proteins, histone-modifying and chromatin-remodeling complexes, and directly by precluding the recruitment of DNA binding proteins. DNA methylation in repetitive and transposable DNA elements is a mechanism that protects chromosomal integrity by preventing the expression of endoparasitic sequences, which can cause chromosomal instability, translocation and gene disruption. Most gene bodies contain multiple repetitive and transposable elements that are extensively methylated. However, here methylation is positively correlated with gene expression instead of repression, i.e. transcriptional elongation efficiency and suppression of spurious initiation of transcription (Jones 2012, Portela & Esteller 2010). This indicates that the effect of methylation is dependent on genomic and cellular context (Jones 2012).

2.2. LINE-1 and D4Z4 repeat sequences

The human genome contains millions of copies of transposable elements and other repetitive sequences. These non-coding repeat sequences constitute two-thirds of the human genome (de Koning *et al.* 2011). Non-coding DNA, once thought to be ‘junk DNA’, was recently assigned some sort of biochemical function, without clear specification (Dunham *et al.* 2012). Transposons, also known as mobile DNAs or ‘jumping genes’ fall into two different classes: DNA transposons which move by a cut-and-paste mechanism, and retrotransposons which move by a copy-and-paste mechanism through an RNA intermediate. Retrotransposons are subdivided into those sequences that contain Long Terminal Repeats (LTR) and those that do not (non-LTR).

Non-LTR subclass, the only element thought to be currently active in humans, is further divided into LINE-1, *Alu* and SINE (Hancks & Kazazian 2012).

2.2.1. LINE-1 retrotransposons

A substantial fraction of the human genome, more than 30%, is derived directly or indirectly from LINE-1 retrotransposon activity (Hancks & Kazazian 2012). LINE-1 elements with more than half-a-million copies comprise approximately 17% of the human genome (Lander *et al.* 2001). Only 80-100 active full-length LINE-1s are retrotranspositionally and transcriptionally competent while most are inactive due to truncation at 5' ends, point mutations or rearrangements (Hancks & Kazazian 2012). A full-length LINE-1 is approximately 6 kb long and contains a promoter located in the 5'untranslated region, two open reading frames and a poly(A) tail necessary for retrotransposition (Beck *et al.* 2011). LINE-1 mediated retrotransposition has been associated with various human diseases including cancer of different sites (Belancio *et al.* 2008). LINE-1s contribute to cancer and other diseases by causing mutations, insertions, double strand breaks and genome instability. Additionally, LINE-1 insertional mutagenesis can also affect the genome globally by altering the epigenetic landscape and creating genomic structural variants (Beck *et al.* 2011, Belancio *et al.* 2008). For these reasons, the host genome developed a mechanism to defend itself from retrotransposon activity primarily by DNA methylation for repression of LINE-1 expression. The internal promoter used by LINE-1 elements encompasses a CpG island, and they are typically highly methylated (Belancio *et al.* 2008). Malignant cells and cancer tissues containing LINE-1 retrotransposition events exhibit hypomethylation of the LINE-1 5'untranslated region and global patterns of hypomethylation, providing a correlative link between epigenetic changes and increased LINE-1 retrotransposition in tumors (Beck *et al.* 2011). Because they reflect the global patterns of methylation, LINE-1 and other non-LTR repetitive sequences have been used as surrogate measure of cellular global DNA methylation (Estecio *et al.* 2007, Yang *et al.* 2004). LINE-1 methylation can be quantified using simple assays with small amount of DNA making it suitable for large-scale global DNA methylation profiling in epigenetic epidemiology and integrated molecular pathological epidemiology studies (Terry *et al.* 2011, Ogino *et al.* 2013).

2.2.2. D4Z4 tandem repeats

The tandem DNA repeat D4Z4 is a subtelomeric macrosatellite repeat array located approximately 40-60 kb proximal to the telomere repeats in chromosomes 4q, 10q and all acrocentric chromosomes. The D4Z4 repeat unit is 3.3 kb long and multiple units are ordered head-to-tail to form the D4Z4 tandem repeat array (Lyle *et al.* 1995, van der Maarel *et al.* 2011). The D4Z4 repeat on chromosome 4, almost identical to that of chromosome 10, contains between 11 and over 100 copies of D4Z4 units (van der Maarel *et al.* 2011). Every repeat unit contains a retrotransposed gene called double homeobox 4 (*DUX4*), a transcription factor that has been shown to regulate several genes involved in various cellular processes, including oncogenesis, immune function, and apoptosis (Geng *et al.* 2012). D4Z4 repeats have high CpG content, and aberrant methylation changes have been observed in different human diseases in addition to cancer (Choi *et al.* 2009, Ehrlich *et al.* 2007, Fraga *et al.* 2005b, van der Maarel *et al.* 2012).

2.3. Methods and techniques to study DNA methylation

Measuring the pattern and distribution of genomic DNA methylation is important to understand its function and explore its association with disease. DNA methylation can be quantified at different levels of the genome, by means of global DNA methylation, locus specific DNA methylation, and genome-wide methylation studies. The details of currently available techniques and methods with their advantages and disadvantages have been recently revised (Lara *et al.* 2011). One relatively novel method used for quantification of DNA methylation is pyrosequencing. Pyrosequencing is a sequencing-by-synthesis method that measures the degree of methylated cytosine by using bisulfite-modified DNA. In this method, nucleotides complementary to the template DNA are sequentially added and incorporated by a DNA polymerase. This process releases pyrophosphate molecules that are quantitatively converted into bioluminometric signal characteristic to each nucleotide incorporated. Bisulfite treatment converts unmethylated cytosine to uracil (thymine after PCR amplification) while methylated cytosine are refractory to the treatment. The level of methylation at each CpG position is then expressed (%) as the ratio of methylated cytosine over the sum of total thymine and methylated cytosine. Methylation values for any CpG position analyzed are given in a pyrosequencing out called pyrogram. Examples of a typical pyrogram for LINE-1 and D4Z4 sequences are shown in

Figure 1-1 and Figure 1-2, respectively. Pyrosequencing, which is being widely used in molecular epidemiology studies, offers the ability of direct quantitative sequencing, accurate and reproducible measurements, speed and ease of use (Tost & Gut 2007, Laird 2010, Lara *et al.* 2011).

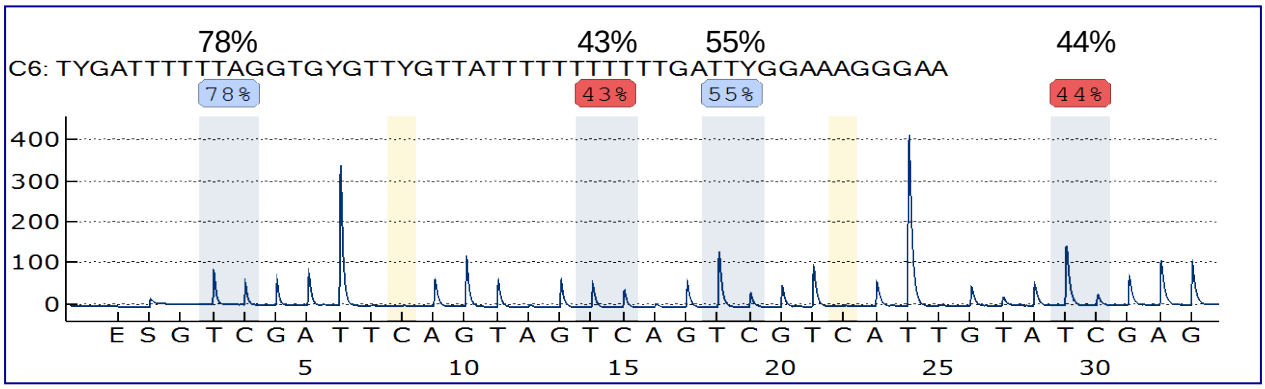

Figure 1-1 A typical pyrogram analyzing four CpG sites of LINE-1 sequences and their respective methylation level. The y-axis represents the relative light signal intensity while the x-axis shows the nucleotide dispensation order. Blue columns indicate the measured CpG sites while yellow columns indicate controls of bisulfite treatment.

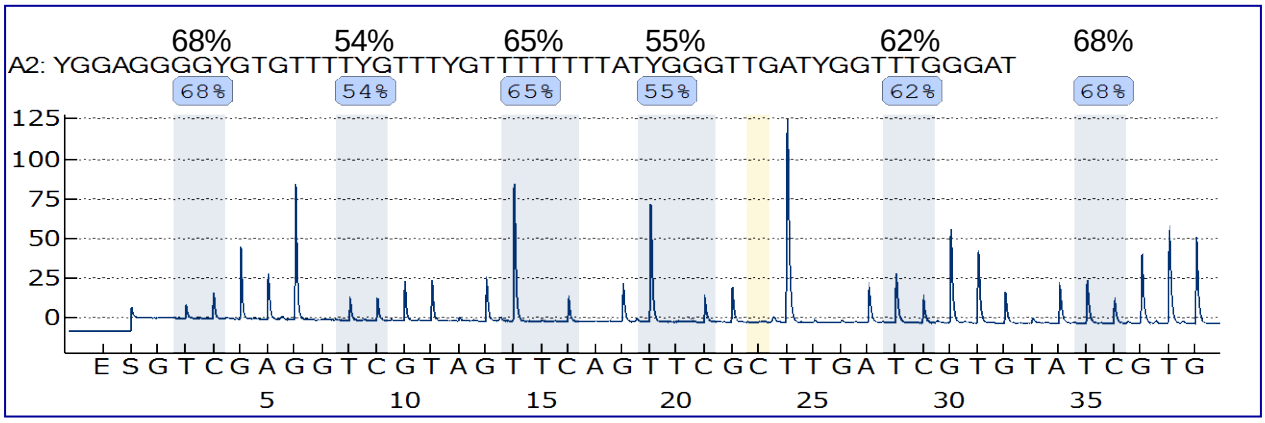

Figure 1-2 A typical pyrogram analyzing six CpG sites of D4Z4 sequences and their respective methylation level. The y-axis represents the relative light signal intensity while the x-axis shows the nucleotide dispensation order. Blue columns indicate the measured CpG sites while yellow columns indicate controls of bisulfite treatment.

2.4. Predictors of genomic DNA methylation

DNA methylation changes over time in response to cellular and environmental cues. These are broadly classified as extrinsic (environmental), intrinsic (genetic), and stochastic factors (Fraga 2009). Altered DNA methylation has been implicated as mechanism through which these factors contribute in the pathogenesis and development of common complex diseases. Intrinsic factors such as sex have been linked to DNA methylation. External factors include life-style behaviors (smoking, physical activity), nutritional factors, and chemical pollutants (arsenic, particulate matters, benzene) (Terry *et al.* 2011). Many dietary components including folate, B vitamins, methionine and choline have been associated with DNA methylation. These nutrients are essential cofactors in the one-carbon metabolism pathway that generate *S*-adenosylmethionine, which is the methyl group donor for the DNA methylation reaction (Feil & Fraga 2011). Genetic factors are also known to modulate DNA methylation. Common genetic variants in one of the one-carbon metabolism genes (*MTHFR*) have been reported to be predictors of genomic DNA methylation (Friso *et al.* 2002, Castro *et al.* 2004). Factors associated with DNA methylation (global and at specific repetitive elements other than LINE-1) have been reviewed elsewhere (Terry *et al.* 2011). Table 1-1 summarizes factors that have been studied so far in relation to LINE-1 methylation levels in peripheral blood cells (Alegria-Torres *et al.* 2013, Baccarelli *et al.* 2009, Bollati *et al.* 2007, Bollati *et al.* 2009, Cash *et al.* 2011, Cash *et al.* 2012, Chalitchagorn *et al.* 2004, El-Maarri *et al.* 2007, El-Maarri *et al.* 2011, Figueiredo *et al.* 2009, Flom *et al.* 2011, Fryer *et al.* 2009, Hsiung *et al.* 2007, Hubner *et al.* 2013, Kim *et al.* 2010, Li *et al.* 2013, Lumey *et al.* 2012, Madrigano *et al.* 2011, Peluso *et al.* 2012, Pilsner *et al.* 2009, Piyathilake *et al.* 2013, Rusiecki *et al.* 2008, Seow *et al.* 2012, Sordillo *et al.* 2012, Subramanyam *et al.* 2013, Tarantini *et al.* 2009, Tehranifar *et al.* 2013, Wilhelm *et al.* 2010, Wright *et al.* 2010, Wu *et al.* 2011b, Zhang *et al.* 2011, Zhu *et al.* 2012).

Table 1-1 Predictors of LINE-1 methylation in leukocyte DNA reported in the literature

Parameters	Direction of association		
	Positive/Direct	**Negative/Inverse**	**Null**
Demographic/Intrinsic			
Age	Subramanyam *et al.* 2013	Bollati *et al.* 2009	Chalitchagorn *et al.* 2004; El-Maarri *et al.* 2011; Hsiung *et al.* 2007; Kim *et al.* 2010; Wilhelm *et al.* 2010; Zhang et al 2011; Zhu *et al.* 2012
Sex (female vs. male)		Cash *et al.* 2011; Cash *et al.* 2012; El-Maarri *et al.* 2007; El-Maarri *et al.* 2011; Hsiung *et al.* 2007; Subramanyam *et al.* 2013; Wilhelm *et al.* 2010; Zhang *et al.* 2011; Zhu *et al.* 2012	Kim *et al.* 2010
Socioeconomic status (wealth)		Subramanyam *et al.* 2013	Tehranifar *et al.* 2013
Life-style behaviors			
Body mass index		Piyathilake *et al.* 2013	Kim *et al.* 2010;Zhang *et al.* 2011; Zhu *et al.* 2012
Physical activity	Zhang *et al.* 2011		
Cigarette smoking			Figueiredo *et al.* 2009; Hsiung *et al.* 2007; Kim *et al.* 2010; Rusieki *et al.* 2008; Wilhelm *et al.* 2010; Zhu *et al.* 2012
Prenatal tobacco smoking			Flom *et al.* 2011

Table 1-1 (cont.) Predictors of LINE-1 methylation in leukocyte DNA reported in the literature

Parameters	Direction of association		
	Positive/Direct	**Negative/Inverse**	**Null**
Alcohol consumption			Hsiung *et al.* 2007; Kim *et al.* 2010; Zhang *et al.* 2011; Zhu *et al.* 2010
Dietary factors			
Prenatal famine exposure			Lumey *et al.* 2012
Folate			Fryer *et al.* 2009; Hübner *et al.* 2013; Hsiung *et al.* 2007; Zhang *et al.* 2011
Vitamin B_{12}			Hübner *et al.* 2013
Vitamin D			Hübner *et al.* 2013
Homocysteine		Fryer *et al.* 2009	
Environmental factors			
Trace elements			
Arsenic		Wilhelm *et al.* 2010	
Lead		Li *et al.* 2013; Pilsner *et al.* 2009; Wright *et al.* 2010	
Air pollutants			
Air pollutants ($PM_{2.5}$, PM_{10})		Baccarelli et al. 2009; Madrigano et al. 2011; Tarantini *et al.* 2009	
Black carbon		Baccarelli *et al.* 2009; Madrigano *et al.* 2011	

Table 1-1 (cont.) Predictors of LINE-1 methylation in leukocyte DNA reported in the literature

Parameters	Direction of association		
	Positive/Direct	**Negative/Inverse**	**Null**
Sulfate		Madrigano *et al.* 2011	
Benzene		Bollati *et al.* 2007	
S-phenylmercapturic acid*		Seow *et al.* 2012	
Trans,trans-muconic acid*			Seow *et al.* 2012
Persistent organic pollutants		Rusieki *et al.* 2008	Kim *et al.* 2010
Polycyclic aromatic hydrocarbons		Peluso *et al.* 2012	Alegría-Torres *et al.* 2013
Genetic factors			
DNA adduct - M_1dG		Peluso *et al.* 2012	
Medical conditions			
Family history of cancer		Wu *et al.* 2011	
Allergen sensitization			Sordillo *et al.* 2012

Abbreviations: PM, particulate matter (number indicate aerodynamic diameter); M_1dG, 3-(2-deoxy-b-D-erythropentafuranosyl)pyrimido[1,2-a]purin-10(3H)-one deoxyguanosine
*Urinary biomarkers of benzene metabolism

2.5. DNA methylation in health and disease

Perturbations in DNA methylation have been shown to play a role in pathogenesis and progression of common complex diseases such as cancer, diabetes, hypertension, autoimmune diseases, and neuropsychiatric disorders (Heyn & Esteller 2012, Baylin & Jones 2011, Portela & Esteller 2010, Robertson 2005, Feinberg & Tycko 2004). However, it is in cancer where altered DNA methylation has been extensively studied and is considered a typical hallmark of the disease (Fernandez *et al.* 2012). Aberrant DNA methylation, an early event in cancer development, is thought to provide the second hit for cancer initiation, postulated by the two-hit model, by inactivating the remaining active allele (Rodriguez-Paredes & Esteller 2011). Over the last several decades, accumulating evidence shows that cancer cells display decreased DNA methylation globally and at promoters of oncogenes, and regional hypermethylation at promoters of tumor suppressor genes (Heyn & Esteller 2012, Taby & Issa 2010, Esteller 2008). Global loss of DNA methylation in cancer tissues is usually found in repetitive elements (LINE-1, *Alu*) and usually contributes to tumor development through promotion of genomic instability and reactivation of transposable elements (Taby & Issa 2010, Esteller 2008). In an effort to find a susceptibility biomarker for cancer that could potentially be used for cancer screening and diagnosis, DNA methylation at peripheral blood cells has been used in epigenetic epidemiology studies. Recent reviews by Woo *et al.* and Brennan *et al.* have shown that global hypomethylation in peripheral blood cells might be associated with increased risk of cancer at several sites (Brennan & Flanagan 2012, Woo & Kim 2012). However, this association seems to be inconsistent when the association between cancer risk and surrogate measures of global DNA methylation is used. This inconsistency was attributed to heterogeneity of the techniques used to quantify DNA methylation (Brennan & Flanagan 2012, Woo & Kim 2012).

2.6. DNA methylation and risk of urothelial carcinoma of the bladder

Studies conducted so far were able to identify important risk factors that can lead to the development of UCB. However, these studies have not fully elucidated its etiology. Accumulating evidence suggests that epigenetic alterations may provide an additional explanation of UCB etiology and pathophysiology. Studies conducted in bladder tumor tissues and cell lines have shown alterations in DNA methylation patterns. These altered DNA

methylation patterns include promoter hypermethylation of specific genes such as *APC*, *DAPK1*, *CDH1*, *RASSF1A* and hypomethylation of repetitive DNA sequences (Kim & Kim 2012, Heyn & Esteller 2012). Furthermore, using DNA methylation analyses of thousands of CpG sites in urothelial carcinoma, Lauss *et al.* were able to define distinct epigenetic subtypes of UCB. Interestingly, non-Island CpGs were overrepresented among the CpG sites that defined these epigenetic subtypes (Lauss *et al.* 2012). An earlier study by Jurgens *et al.* and a follow-up study by Florl *et al.* showed that LINE-1 retrotransposon in tumor tissue, compared to normal bladder tissue, presents low levels of methylation (Jurgens *et al.* 1996, Florl *et al.* 1999).

Similar to what has been observed in UCB tissue, decreased global DNA methylation level in peripheral blood cell DNA has been associated with increased risk of developing the disease (Moore *et al.* 2008, Wilhelm *et al.* 2010, Cash *et al.* 2012). The latter two studies used pyrosequencing of LINE-1 methylation as a surrogate marker of genomic DNA methylation, while the first study by Moore *et al.* measured the total 5-methylcytosine content in the genome by high-performance capillary electrophoresis. The summary results of these studies are shown in Table 1-2.

Table 1-2 Association between peripheral blood DNA methylation and risk of urothelial carcinoma of the bladder

First author, Year	**Study design**	**Sample size**	**Assay type**	**Odds ratio**	***P***	**Remark**
Moore *et al.* 2008	Case-control	775 cases and 397 controls	*HpaII*/HPCE & densitometry of global 5-methylcytosine	2.67 (lowest versus highest quartile)	—	DNA methylation-smoking interaction
Wilhelm *et al.* 2010	Case-control	285 cases and 465 controls	Pyrosequencing of LINE-1	1.80 (lowest decile versus the rest)	0.02	no interaction was observed
Cash *et al.* 2012	Case-control	510 cases and 528 controls	Pyrosequencing of LINE-1	1.91 (lowest versus highest tertile among never smokers)	0.03	DNA methylation-*GSTM1*/*GSTT1* interaction

HPCE, high-performance capillary electrophoresis

2.7. DNA methylation and prognosis of urothelial carcinoma of the bladder

It has been suggested that a molecular-guided approach would supplement and improve the current prognostic scoring system. One interesting area to identify novel molecular biomarkers is the study of DNA methylation in different genomic regions. Studies conducted in tumor tissue have reported altered DNA methylation in gene promoters as potential prognostic markers for both NMIBC and MIBC. These include promoter hypermethylation of *RASSAF1*, *DAPK*, *APC* and *EDNRB* in NMIBC and *RASSF1*, *CDH1* and *EDNRB* in MIBC (Netto 2012). Alterations in promoter methylation levels have been associated with tumor stage and grade, increased recurrence, progression and mortality (Mitra & Cote 2009, Kim & Kim 2012). On the other hand global hypomethylation in tumor tissue has been shown to associate with significantly shorter time to recurrence and disease-specific survival time (Neuhausen *et al.* 2006). So far, no study has assessed the association between peripheral blood cell DNA methylation and UCB outcomes.

3. Case for action

Urothelial carcinoma of the bladder is one of the commonest cancers and is associated with increased morbidity and mortality. Because surveillance and frequent follow-ups are needed, often using expensive and invasive tests and imaging techniques, UCB is one of the most costly cancers to treat. Biomarkers contribute to cancer control and prevention by identifying high-risk pre-cancerous lesions and detection of cancer at its early stage. Epigenetic modifications such as DNA methylation can provide a link between genetic and environmental factors and phenotypic changes and disease risk. Research on DNA methylation promises to reveal many of the causes of diseases that remain undiscovered after extensive investigation of common genetic variations (Ng *et al.* 2012). Increasing studies report the potential of DNA methylation markers in various biofluids in improving diagnostic and therapeutic efficiency in multiple cancer types (Issa 2012, Nogueira da Costa & Herceg 2012). Hence, studying DNA methylation in leukocyte DNA and its association with UCB risk and prognosis may help identify susceptibility and prognostic biomarkers, which could eventually be used for early detection and risk stratification for individualized and improved patient care. Moreover, results from such study might shade light on the underlying etiology of the disease that could be a potential target for chemoprevention and treatment. Finally, these findings may contribute towards devising evidence-based public health preventive interventions to UCB by identifying a specific group of susceptible subjects who may benefit most from these preventive interventions, thus reducing morbidity and mortality.

Therefore, by using and integrating data from the Spanish Bladder Cancer/EPIdemiology of Cancer of the UROthelium (SBC/EPICURO) study, with this PhD thesis I aimed to study the role of DNA methylation at LINE-1 and D4Z4 repetitive elements on the risk and survival of UCB, and its interaction with environmental and genetic factors.

4. The SBC/EPICURO study

The SBC/EPICURO study is a multicenter hospital-based case-control study conducted in Spain from 1998 to 2001. The study recruited 1219 cases that are newly

diagnosed with urothelial carcinoma of the bladder and 1271 controls from 18 hospitals located in Asturias, Barcelona, Elche, Tenerife, and Valles. Diagnosis of UCB was confirmed by a panel of expert pathologists based on the 1998 World Health Organization/International Society of Urological Pathology classification system. Controls were patients admitted to the same hospital as cases for reasons unrelated to the exposure of interest. Cases were individually matched to controls based on age at interview (five-year interval), gender, geographic region, and smoking status. The age range of study participants was between 19 and 81 years. Data on demographic, known and potential risk factors for UCB, clinicopathological characteristics were collected using computer-assisted personal interviews. The study also collected blood for DNA extraction, and toenail clippings to measure trace elements. Cases were followed yearly for almost 10 years, and data on recurrence, progression and mortality were recorded. In the present thesis, data from 952 cases and 892 controls with LINE-1 methylation measurements, and 707 cases and 718 controls with D4Z4 methylation measurements were included in the analyses.

CHAPTER II. HYPOTHESES AND OBJECTIVES

1. Hypotheses

Some genetic and non-genetic factors are predictors of DNA methylation. Altered genomic DNA methylation level is associated risk of UCB and its clinical outcomes.

2. Objectives

The general objective of this work was to identify predictors of granulocyte DNA methylation and evaluate the role of methylation in UCB risk and outcomes.

The specific objectives were:

1. To identify genetic and non-genetic predictors of LINE-1 and D4Z4 methylation
2. To assess the association between LINE-1 methylation and UCB risk, and its modification by known and potential risk factors
3. To investigate the association between D4Z4 methylation and UCB risk, and its modification by known and potential risk factors
4. To determine the prognostic significance of LINE-1 and D4Z4 methylation in both NMIBC and MIBC outcomes

Each of the following chapters is structured as a scientific manuscript (abstract, introduction, methods, results, discussion, and conclusions) in an attempt to address each of the specific objectives listed above. ***Chapter III*** addresses the predictors of LINE-1 methylation in leukocyte DNA; ***Chapter IV*** discusses the association between LINE-1 methylation and UCB risk, and its modification by known and potential risk factors; ***Chapter V*** addresses the potential predictors of D4Z4 methylation and its role in UCB risk; ***Chapter VI*** describes an assessment of UCB and the prognostic role of LINE-1 and D4Z4

methylation; ***Chapter VII*** presents further integrated discussion of the main results, and lists recommendations for future research to build upon this work in particular; and ***Chapter VIII*** provides the main conclusions drawn from this work.

CHAPTER III. GENETIC AND NON-GENETIC PREDICTORS OF LINE-1 METHYLATION IN LEUKOCYTE DNA

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Environmental Health Perspectives 2013: doi:10.1289/ehp.1206068

Abstract

Altered DNA methylation has been associated with various diseases. To evaluate the association between levels of methylation in leukocyte DNA at long interspersed nuclear element 1 (LINE-1) and genetic and non-genetic characteristics of 892 control participants from the Spanish Bladder Cancer/EPICURO Study. We determined LINE-1 methylation levels by pyrosequencing. Individual data included demographics, smoking status, nutrient intake, toenail concentrations of 12 trace elements, xenobiotic metabolism gene variants, and 515 polymorphisms among 24 genes in the one-carbon metabolism pathway. To assess the association between LINE-1 methylation levels (percentage of methylated cytosines) and potential determinants, we estimated beta coefficients (β) by robust linear regression. Females had lower levels of LINE-1 methylation than males (β=-0.7, p-value=0.02). Blond tobacco smokers showed lower methylation than non-smokers (β=-0.7, p-value=0.03). Arsenic toenail concentration was inversely associated with LINE-1 methylation (β=-3.6, p-value=0.003). By contrast, iron (β=0.002, p-value=0.009) and nickel (β=0.02, p-value=0.004) were positively associated with LINE-1 methylation. SNPs in *DNMT3A* (rs7581217-per allele, β=0.3, p-value=0.002), *TCN2* (rs9606756-GG, β=1.9, p-value=0.008; rs4820887-AA, β=4.0, p-value=$4.8x10^{-7}$; rs9621049-TT, β=4.2, p-value=$4.7x10^{-9}$), *AS3MT* (rs7085104-GG, β=0.7, p-value=0.001), *SLC19A1* (rs914238, TC vs TT: β=0.5 and CC vs TT: β=-0.3, global p-value=0.0007) and *MTHFS* (rs1380642, CT vs CC: β=0.3 and TT vs CC; β=-0.8, global p-value=0.05) were associated with LINE-1 methylation. We identified several characteristics, environmental factors, and common genetic variants that predicted DNA methylation among study participants.

Introduction

DNA methylation plays a fundamental role in regulation of gene expression, genomic imprinting, X-chromosome inactivation, and repression of transposable elements (Jones & Liang 2009). Aberrant DNA methylation has been associated with various cancers, and with developmental, autoimmune, and other chronic diseases (Robertson 2005). Global DNA methylation can be directly quantified by measuring 5-methylcytosine content of the genome, or can be estimated based on methylation of repetitive sequences such as Alu elements or long interspersed nuclear element 1 (LINE-1) (Yang et al. 2004). Age, sex, smoking, and arsenic and lead exposures have been associated with DNA methylation, but findings have been inconsistent among studies (Fuke *et al.* 2004, El-Maarri *et al.* 2007, Terry *et al.* 2011, Breitling *et al.* 2011, Fraga *et al.* 2005a). The folate and methionine-dependent one-carbon metabolism pathway could modulate DNA methylation by altering the level of S-adenosylmethionine (SAM), the principal source of methyl groups (Ulrey et al. 2005). Genetic variants might also influence the methylation of CpG loci locally, or might have a global influence on methylation throughout the genome. For example, a single nucleotide polymorphism (SNP) in *TRPC3*-isoform 2 has been reported to regulate the methylation status of its own promoter (Martin-Trujillo et al. 2011), and variants of the methylenetetrahydrofolate reductase gene *(MTHFR)* have been associated with global DNA hypomethylation (Friso *et al.* 2002, Castro *et al.* 2004). However, although the determinants of global and site-specific methylation are widely assumed to be likely contributors to health and disease, they are poorly defined at this time.

Assessing the impact of both genetic and non-genetic factors on global DNA methylation may improve our understanding of the molecular pathogenesis of many common diseases. Therefore, we investigated associations of global DNA methylation in LINE-1 from bisulfite-modified granulocyte DNA with genetic variants and personal, demographic, lifestyle, and environmental characteristics.

Methods

Study population: The study population, design, and data collection have been previously described (Garcia-Closas et al. 2005). Briefly, participating individuals were controls from the Spanish Bladder Cancer/EPICURO Study who were admitted to hospitals in five regions of Spain for a range of conditions including hernia, fractures, and other non-cancer diseases, and were 20–81 years of age. We collected demographic and exposure information at the hospitals using computer-assisted personal interviews. From a total of 1,271 controls that agreed to participate in the study and were interviewed, 1,056 provided blood for DNA extraction. We excluded twenty-three subjects because of inadequate or poor quality DNA (N=15) or missing smoking status data (N=8). Three subjects with missing data on smoking status were included because they had data on other variables including age, gender, region, and body mass index (BMI). To ensure homogeneity, we also excluded one non-Caucasian individual, leaving 925 individuals with granulocyte DNA for bisulfite modification and pyrosequencing. Pyrosequencing failed in 33 individuals; thus, the final study population for the present analysis included 892 participants. We obtained written informed consent from all participants, and the study was approved by the local Spanish institutional review boards and US National Cancer Institute.

Quantification of LINE-1 methylation: We extracted granulocyte DNA using standard methods (Garcia-Closas et al. 2005). We carried out bisulfite conversion of DNA using the EZ-96 DNA METHYLATION-GOLD^{TM} KIT (Zymo Research, Irvine, CA, USA) according to the manufacturer's recommendations. We carried out PCR amplification of bisulfite-modified DNA using a set of forward and reverse primers reported previously (Estecio et al. 2007). To quantify the methylation level of each of the first four CpG sites next to the pyrosequencing primer, we performed sequencing of the PCR product by pyrosequencing, using the PyroMarkTM Q24 System (QIAGEN, Valencia, CA, USA) as recommended by the manufacturer. The first four were the CpGs from which we could obtain methylation values of all samples. We extracted the methylation level at each CpG site using the PyroMarkTM Application Software version 2.0.6 (QIAGEN, Valencia, CA, USA), and we expressed the value as the percentage of methylated cytosines over the sum of methylated and unmethylated cytosines. We used the average methylation level of the first four LINE-1 CpG sites as a surrogate marker of the global DNA

methylation level. To determine whether changes in blood cell populations affect LINE-1 methylation levels, we analyzed LINE-1 methylation in independently purified granulocyte and lymphocyte samples. The results showed no significant difference in LINE-1 methylation between granulocyte and lymphocyte samples, thus suggesting that variation in the distribution of peripheral blood cell populations among participants would not contribute to variation in global DNA methylation (data not shown). For the present study, we used DNA extracted from granulocyte to quantify DNA methylation. As a quality control measure, we measured LINE-1 methylation in 129 randomly selected duplicate samples and the within-sample coefficient of variation (CV) was 4.0%. In the analysis, we used the average of the duplicates for those samples.

Nutritional assessment: We estimated the usual intake of vitamins B_1, B_2, B_3, B_6, and B_{12}, folate, protein, alcohol, fruit and vegetable over the five years before interview using a validated food frequency questionnaire of 127 items (Garcia-Closas *et al.* 2007b). Micronutrients and macronutrients included in the present analysis have been suggested as important co-factors and methyl donors in one-carbon metabolism (Stover 2009). We calculated nutrient density variables by dividing the total estimated mass of daily food consumed by the total estimated daily energy intake (μg/day/kcal).

Trace elements: We collected toenail clippings to estimate chronic exposure to trace elements. Sample collection and experimental methods used to measure trace elements level have been reported (Amaral *et al.* 2012c). Briefly, after cleaning and washing the toenails to remove external contaminants, we quantified elements at the Trace Element Analysis Core (Dartmouth College, NH, USA), using inductively coupled plasma-mass spectrometry (Hopkins et al. 2004). We digested the samples with Optima HNO_3 (Fisher Scientific, St. Louis, MO) at 105 °C followed by addition of H_2O_2 and further heating the dilution with deionized water. We recorded gravimetrically all sample preparation steps. As a quality control, each batch of analyses included six standard reference material samples with known trace element content (SRM; GBW 07601, powdered human hair) and six analytic blanks, along with the study samples. In total, we determined concentrations (μg/g) of 12 trace elements (aluminum, arsenic, cadmium, chromium, copper, iron, lead, manganese, nickel, selenium, vanadium, and zinc).

Genotyping: For genotype assays, we extracted DNA from leukocytes as described previously (Garcia-Closas et al. 2005). We determined genotypes at the Core Genotyping Facility of the Division of Cancer Epidemiology and Genetics, National Cancer Institute, MD, USA. We selected for the analysis a total of 515 SNPs in 24 genes involved in the one-carbon metabolism pathway, including DNA methylation and arsenic metabolism (see Supplementary Table S3-1 for a list of the 24 genes evaluated). We selected these genes because they are critical for the one-carbon metabolism pathway (Ulrey *et al.* 2005, Lee *et al.* 2009). Previously, we described in detail methods of the genotyping process (Garcia-Closas *et al*. 2005, Garcia-Closas *et al.* 2007a, Rothman *et al.* 2010). We genotyped SNPs using Illumina Infinium® Human1M-Duo, Illumina GoldenGate® and TaqMan® assays (see Supplementary Table S3-2 for a complete list of SNPs according to assay). In addition, we estimated associations between LINE-1 methylation and *GSTM1, GSTT1,* and *NAT2* variants because of their relevance to bladder cancer (Cash et al. 2012). These variants were determined as described in (Garcia-Closas et al. 2005). All genotypes included in the study were in Hardy-Weinberg Equilibrium in the study population (p-value > 0.05) (data not shown).

Statistical analysis: The distribution of LINE-1 methylation levels was slightly bimodal and positively skewed (see Supplementary Figure S3-1). To estimate associations between LINE-1 methylation levels and each of the variables considered, we fitted bivariate robust linear regression models and calculated the corresponding beta coefficients and 95% confidence intervals (95%CI). Characteristics analyzed as continuous variables were age, micronutrient intakes, fruit and vegetable intakes, and toenail concentrations of trace elements. Characteristics analyzed as categorical variables were BMI (<25.0, 25.0-26.99, 27.0-29.99, ≥30.0), smoking status (non-, occasional, former, current smoker), and tobacco type (non-smoker, blond only, black only, blond and black, unknown).

To identify SNPs for detailed assessment we first used the Fisher's exact test to screen SNPs that were significantly associated ($p<0.05$) with LINE-1 methylation categorized according to tertiles (<56.7%, 56.7–58.6%, and >58.6%) according to codominant mode of inheritance. The 22 SNPs identified for further analyses (listed in Supplementary Table S3-3) were subsequently modeled

according to all modes of inheritance (additive, codominant, dominant and recessive). The mode of inheritance that best predicted LINE-1 methylation is reported in Table 3-2.

In addition to age and gender, adjusted robust linear regression models for each potential predictor included region, which may be related to diet, micronutrients (Gabriel et al. 2006), and environmental pollution, and smoking status, which may be related to trace elements (Moerman & Potts 2011). We also did a sensitivity analysis without adjusting for smoking status to see if there was a change in the beta estimates. We included all the 515 SNPs in the analysis regardless of linkage disequilibrium. The association between LINE-1 methylation and potential predictors was assessed in the multivariable adjusted model stratifying by gender. Because arsenic and rs7085104 in arsenic (+3 oxidation state) methyltransferase (*AS3MT*), which is involved in arsenic metabolism, were individually associated with LINE-1 methylation, we assessed the presence of effect modification by including a multiplicative interaction term in a model adjusted for age, gender, region and smoking status. Wald test was used to calculate the interaction p-value. We corrected for multiple testing using the Bonferroni's method. We conducted a sensitivity analysis, excluding 42 individuals with CV >4% for LINE-1 methylation in duplicate samples. This exclusion did not result in substantial differences in the beta coefficients; therefore these individuals remained in the analyses (data not shown). All statistical tests were two-sided, and a p-value ≤ 0.05 was considered significant. We carried all data analyses ou t using STATA/SE version 10.1 (StataCorp, College Station, TX, USA).

Results

The characteristics of the 892 participants in the present study and median and mean LINE-1 methylation levels according to each variable of interest are provided in Supplementary Table S3-4. The majority of the study participants were males (89%) and regular smokers (63.9%), with median age of 66 years [interquartile range (IQR)=13]. The mean LINE-1 methylation level was 58.9% (SD=5.3%) with minimum and maximum value of 37.9% and 85.7%, respectively. Table 3-1 shows the association between LINE-1 methylation levels and characteristics of study subjects. In the bivariable robust linear regression analysis, only toenail arsenic and nickel concentrations were significantly associated with LINE-1 methylation. However, in multivariable robust linear regression models adjusted for age, gender, region, and smoking status, the levels of LINE-1 methylation were significantly lower in females than in males (adjusted β = -0.7; 95%CI: -1.2, -0.1, p-value=0.02) and in smokers of blond tobacco only (adjusted β = -0.7; 95%CI: -1.3, -0.08, p-value=0.03) and of both blond and black tobacco (adjusted β = -0.6; 95%CI: -1.1, -0.07, p-value=0.03) compared with nonsmokers. Toenail arsenic concentration also was negatively associated with LINE-1 methylation (adjusted β for a 1-µg/g increase = -3.6; 95%CI: -5.9, -1.2, p-value=0.003). In contrast, LINE-1 levels were positively associated with 1-µg/g increases in toenail concentrations of iron (adjusted β = 0.002; 95%CI: 0.001, 0.004, p-value=0.009) and nickel (adjusted β = 0.02; 95%CI: 0.005, 0.03, p-value=0.004). BMI, B vitamins, folate, total protein, alcohol, fruit and vegetable intake were not significantly associated with LINE-1 methylation regardless of adjustment for covariates (Table 3-1). Results from the multivariable analyses without adjusting for smoking status done as a sensitivity analysis were not different from the associations above (see Supplementary Table S3-5). If we were to correct for multiple comparisons by Bonferroni's method none of the above would be significant.

Table 3-1 Association between LINE-1 methylation level and individual characteristics of study subjects in the SBC/EPICURO Study

Variables	N	LINE-1 methylation Mean (95% CI)	Unadjusted β (95% CI)	P-value	N	Adjusted β(95% CI)*†	P-value
Age (years)	892	—	-0.004 (-0.02, 0.01)	0.6	889	-0.006 (-0.02, 0.01)	0.5
Gender							
Male	792	59.0 (58.6, 59.4)	Reference		789	Reference	
Female	100	58.0 (57.1, 58.8)	-0.4 (-0.9, 0.05)	0.08	100	-0.7 (-1.2, -0.1)	0.02
Region							
Barcelona	168	59.3 (58.5, 60.2)	Reference		168	Reference	
Valles	135	58.3 (57.5, 59.0)	-0.2 (-0.7, 0.4)	0.5	135	-0.2 (-0.7, 0.3)	0.5
Elche	73	58.2 (57.1, 59.3)	-0.4 (-1.1, 0.2)	0.2	73	-0.5 (-1.1, 0.2)	0.1
Tenerife	145	58.6 (57.8, 59.4)	-0.1 (-0.7, 0.4)	0.7	144	-0.1 (-0.6, 0.4)	0.7
Asturias	371	59.2 (58.6, 59.8)	0.2 (-0.3, 0.6)	0.5	369	0.1 (-0.3, 0.6)	0.6
Body mass index (kg/m²)							
<25.0	372	58.8 (58.3, 59.3)	Reference		370	Reference	
25.0-26.99	148	58.9 (57.9, 59.9)	-0.2 (-0.6, 0.3)	0.5	148	-0.2 (-0.6, 0.3)	0.4
27.0-29.99	120	59.0 (58.1, 59.8)	0.1 (-0.3, 0.6)	0.6	120	0.2 (-0.3, 0.7)	0.4
≥30.0	57	58.8 (57.5, 60.2)	-0.08 (-0.7, 0.6)	0.8	57	-0.03 (-0.7, 0.6)	0.9
Missing data	195						
Smoking status							
Non-smoker	255	58.2 (57.7, 58.7)	Reference		255	Reference	
Occasional smoker	66	60.0 (58.4, 61.5)	0.4 (-0.2, 1.1)	0.2	66	0.3 (-0.3, 1.0)	0.3
Former smoker	329	58.9 (58.3, 59.5)	-0.2 (-0.5, 0.3)	0.6	329	-0.3 (-0.7, 0.1)	0.2
Current smoker	239	59.4 (58.6, 60.2)	-0.2 (-0.6, 0.2)	0.4	239	-0.4 (-0.9, 0.07)	0.1
Missing data	3						
Tobacco type							
Non-smoker	255	58.2 (57.7, 58.7)	Reference		255	Reference	
Blond tobacco only	99	58.5 (57.6, 59.5)	-0.5 (-1.0, 0.09)	0.1	99	-0.7 (-1.3, -0.08)	0.03
Black tobacco only	219	59.6 (58.8, 60.4)	0.06 (-0.4, 0.5)	0.8	218	-0.2 (-0.6, 0.3)	0.5
Blond and black	154	58.7 (57.8, 59.6)	-0.3 (-0.8, 0.2)	0.2	154	-0.6 (-1.1, -0.07)	0.03
Unknown	97	59.3 (58.2, 60.5)	-0.01 (-0.6, 0.6)	0.9	97	-0.12 (-0.7, 0.5)	0.7
Missing data	68						

Table 3-1 (cont.) Association between LINE-1 methylation level and individual characteristics of study subjects in the SBC/EPICURO Study

Variables	N	LINE-1 methylation Mean (95% CI)	Unadjusted β (95% CI)	P-value	N	Adjusted β(95% CI)*†	P-value
Controls' diagnosis							
Hernia	332	58.8 (58.2, 59.4)	Reference		330	Reference	
Fracture & Trauma	263	59.3 (58.6, 60.0)	-0.2 (-0.5, 0.2)	0.4	262	-0.02 (-0.4, 0.4)	0.9
Hydrocele	122	58.7 (57.8, 59.6)	0.2 (-0.3, 0.7)	0.4	122	0.2 (-0.3, 0.7)	0.5
Other Abdominal Surgery	99	58.3 (57.4, 59.2)	-0.2 (-0.7, 0.3)	0.4	99	-0.09 (-0.6, 0.5)	0.7
Other Diseases	76	59.2 (57.9, 60.5)	0.01 (-0.9, 0.6)	0.9	76	0.2 (-0.4, 0.8)	0.5
Dietary intake‡							
Vitamin B_1 (µg/day/kcal)	645	–	0.5 (-0.6, 1.6)	0.3	644	0.6 (-0.6, 1.7)	0.3
Vitamin B_2 (µg/day/kcal)	645	–	0.1 (-0.5, 0.8)	0.7	644	0.2 (-0.5, 0.8)	0.6
Vitamin B_3 (µg/day/kcal)	645	–	0.01 (-0.05, 0.08)	0.7	644	0.02 (-0.05, 0.09)	0.5
Vitamin B_6 (µg/day/kcal)	645	–	0.6 (-0.2, 1.4)	0.1	644	0.8 (-0.05, 1.6)	0.07
Vitamin B_{12} (µg/day/kcal)	645	–	-0.04 (-0.09, 0.01)	0.1	644	-0.03 (-0.08, 0.02)	0.3
Folate (µg/day/kcal)	645	–	0.001 (-0.002, 0.004)	0.4	644	0.003 (-0.001, 0.01)	0.1
Protein (µg/day/kcal)	645	–	0.01 (-0.01, 0.03)	0.4	644	0.01 (-0.01, 0.03)	0.4
Alcohol (µg/day/kcal)	645	–	-0.002 (-0.02, 0.02)	0.8	644	-0.01 (-0.03, 0.02)	0.5
Fruit (g/day/kcal)	639	–	0.0001 (-0.001, 0.002)	0.9	638	0.0001 (-0.001, 0.002)	0.9
Vegetable (g/day/kcal)	640	–	0.001 (-0.001, 0.003)	0.3	639	0.002 (-0.001, 0.004)	0.1
Fruit and vegetable (g/day/kcal)	639	–	0.0003 (-0.0008, 0.001)	0.6	638	0.0005 (-0.0007, 0.002)	0.4
Toenail trace elements§							
Aluminum (µg/g)	658	–	-0.003 (-0.008, 0.002)	0.2	658	-0.003 (-0.008, 0.002)	0.2
Arsenic (µg/g)	659	–	-2.9 (-5.2, -0.6)	0.02	659	-3.6 (-5.9, -1.2)	0.003
Cadmium (µg/g)	659	–	0.08 (-0.4, 0.5)	0.7	659	0.1 (-0.3, 0.6)	0.6
Chromium (µg/g)	658	–	0.06 (-0.01, 0.1)	0.09	659	-0.01 (-0.05, 0.03)	0.6
Copper (µg/g)	659	–	-0.002 (-0.06, 0.05)	0.95	659	-0.01 (-0.07, 0.04)	0.6
Iron (µg/g)	657	–	-0.002 (-0.006, 0.002)	0.4	658	0.002 (0.001, 0.004)	0.009
Lead (µg/g)	659	–	-0.05 (-0.1, 0.03)	0.2	659	-0.06 (-0.1, 0.02)	0.2
Manganese (µg/g)	659	–	-0.03 (-0.1, 0.09)	0.7	659	-0.05 (-0.2, 0.06)	0.4
Nickel (µg/g)	659	–	0.02 (0.006, 0.03)	0.002	659	0.02 (0.005, 0.03)	0.004
Selenium (µg/g)	659	–	0.1 (-0.8, 1.0)	0.8	659	0.2 (-0.7 1.2)	0.6
Vanadium (µg/g)	651	–	-0.7 (-2.7, 1.3)	0.5	651	-0.9 (-2.8, 1.2)	0.4
Zinc (µg/g)	659	–	-0.002 (-0.004, 0.001)	0.2	659	-0.001 (-0.004, 0.002)	0.4

Table 3-1 (cont.) Association between LINE-1 methylation level and individual characteristics of study subjects in the SBC/EPICURO Study

Variables	N	LINE-1 methylation Mean (95% CI)	Unadjusted β (95% CI)	P-value	N	Adjusted β(95% CI)*†	P-value
NAT2 phenotype							
Rapid/Intermediate acetylator	389	59.0 (58.4, 59.6)	Reference		388	Reference	
Slow acetylator	498	58.8 (58.4, 59.2)	0.2 (-0.1, 0.5)	0.3	496	0.2 (-0.1, 0.5)	0.2
Missing data	5						
GSTM1 genotype							
(+/+, +/-)	421	58.9 (58.3, 59.4)	Reference		419	Reference	
(-/-)	462	59.0 (58.5, 59.4)	0.04 (-0.3, 0.4)	0.8	461	0.01 (-0.3, 0.3)	0.9
Missing data	9						
GSTT1 genotype							
(+/+, +/-)	688	59.0 (58.6, 59.4)	Reference		685	Reference	
(-/-)	198	58.5 (57.9, 59.2)	-0.2 (-0.6, 0.2)	0.3	198	-0.2 (-0.6, 0.2)	0.4
Missing data	6						

*Adjusted for age, gender, region, and smoking status (non-, occasional, former, current smoker). Tobacco type's β is not adjusted for smoking status.

†The number of observations are reduced by three because of missing data on smoking status.

‡Data available for those who completed food frequency questionnaire.

§Data available for those who provided toenail for trace element assessment.

Note: the exposure contrast for trace elements is 1-µg/g and for dietary variables is 1-µg/day/kcal.

Out of the 515 genetic variants assessed, 22 passed a first screening using Fisher's exact test (p-value≤0.05 according to codominant mode of inheritance) (see Supplementary Table S3-3). Of these, seven SNPs in five genes were significantly associated with LINE-1 methylation based on multivariable models adjusted for age, gender, region, and smoking status (Table 3-2; model-based estimates for the 15 SNPs that were not significantly associated with LINE-1 methylation are reported in Supplementary Table S3-6.) Significant positive associations were estimated for *DNMT3A*-rs7581217 (per allele: adjusted β = 0.3; 95%CI: 0.1, 0.6, p-value=0.002)*; TCN2*-rs9621049 (recessive: adjusted β = 4.2; 95%CI: 2.8, 5.7, p-value=$4.7x10^{-9}$), *TCN2*-rs4820887 (recessive: adjusted β = 4.0; 95%CI: 2.5, 5.6, p-value=$4.8x10^{-7}$) and *TCN2*-rs9606756 (recessive: adjusted β = 1.9; 95%CI: 0.5, 3.3, p-value=0.008)*;* and *AS3MT*-rs7085104 (recessive: adjusted β = 0.7; 95%CI: 0.3, 1.2, p-value=0.001). In addition, significant associations under the codominant mode of inheritance (based on global p-values) were estimated for *SLC19A1*-rs914238 (TC vs TT, β = 0.5; 95%CI: 0.08, 0.8; CC vs TT, β = -0.3; 95%CI: -0.7, 0.2; global p-value=0.0007) and *MTHFS*-rs1380642 (CT vs CC, β = 0.3; 95%CI: -0.08, 0.6; TT vs CC, β = -0.8; 95%CI: -1.6, 0.09; global p-value=0.05). After correcting for multiple testing using the Bonferroni's method, *TCN2*-rs9621049 and *TCN2*-rs4820887 remained significant (p<0.05).

Table 3-2 Association between LINE-1 methylation levels and single nucleotide polymorphisms in genes involved in the one-carbon metabolism pathway

Gene	dbSNP [Chromosome, position in the gene, location*]	MAF	N	MOI	Genotype	Unadjusted β (95% CI)	P-value	Adjusted β (95% CI)†	P-value
DNMT3A	rs7581217 [2, intron, 25378448]	0.39	875	Additive	per allele T	0.3 (0.1, 0.6)	0.003	0.3 (0.1, 0.6)	0.002
AS3MT	rs7085104 [10, flanking 5'UTR, 104618863]	0.38	751	Recessive	AA/AG	Reference		Reference	
			124		GG	0.8 (0.3, 1.2)	0.0008	0.7 (0.3, 1.2)	0.001
MTHFS‡	rs1380642 [15, flanking 3'UTR, 77883926]	0.18	585	Codominant	CC	Reference	0.03	Reference	0.05
			258		CT	0.3 (-0.05, 0.6)		0.3 (-0.08, 0.6)	
			32		TT	-0.8 (-1.6, 0.07)		-0.8 (-1.6, 0.09)	
SLC19A1‡	rs914238 [21, flanking 5'UTR, 45840089]	0.49	231	Codominant	TT	Reference	0.0008	Reference	0.0007
			435		TC	0.5 (0.09, 0.8)		0.5 (0.08, 0.8)	
			209		CC	-0.2 (-0.7, 0.2)		-0.3 (-0.7, 0.2)	
TCN2§	rs9621049 [22, exon, 29343419]	0.11	864	Recessive	CC/CT	Reference		Reference	
			11		TT	4.5 (3.1, 5.9)	4.3×10^{-10}	4.2 (2.8, 5.7)	4.7×10^{-9}
	rs9606756 [22, exon, 29336860]	0.12	864	Recessive	AA/AG	Reference		Reference	
			11		GG	2.2 (0.8, 3.6)	0.003	1.9 (0.5, 3.3)	0.008
	rs4820887 [22, intron, 29346914]	0.1	866	Recessive	GG/GA	Reference		Reference	
			9		AA	4.6 (3.0, 6.2)	9.3×10^{-9}	4.0 (2.5, 5.6)	4.8×10^{-7}

MAF, minor allele frequency; MOI, mode of inheritance.

*Human Genome Build 36.3 location.

†Adjusted for age, gender, region, and smoking status.

‡Global p-value for rs1380642 and rs914238 was estimated by using a two-degrees of freedom likelihood-ratio test.

§Linkage disequilibrium (rs4820887 vs. rs9621049) $r^2 = 0.8$.

A significant interaction (p-value=0.01) was observed between arsenic and *AS3MT*-rs7085104 on LINE-1 methylation (adjusted β for 1-μg/g increase in As = -4.1; 95%CI: -6.6, -1.7, p-value=0.001 for genotype AA/AG; and adjusted β for 1-μg/g increase in As = 10.2; 95%CI: -3.2, 23.7, p-value=0.1 for genotype GG).

After simultaneously adjusting for age, geographic region, and all factors that were significant predictors of LINE-1 methylation (sex; tobacco type; toenail arsenic, iron and nickel; and the 5 SNPs noted above), associations with sex, arsenic, nickel, iron, *DNMT3A*-rs7581217, *TCN2*-rs9621049, and *MTHFS*-rs1380642 remained significant. The association with blond tobacco was nonsignificant although the direction of the point estimate remained unchanged. The association between rs9606756, rs4820887 and LINE-1 methylation become nonsignifcant (see Supplementary Table S3-7). This might be due to reduced sample size in the simultaneously adjusted model due to missing data.

Discussion and conclusions

In the present study, we took a comprehensive approach to assess associations of genetic and non-genetic factors with LINE-1 methylation in a group of individuals aged 20-81 years. Lower levels of LINE-1 methylation were found among females compared with males, and among smokers of blond tobacco compared with non-smokers. In addition, toenail concentrations of arsenic were also negatively associated with LINE-1 methylation. On the other hand, LINE-1 methylation levels were positively associated with toenail concentrations of iron and nickel, and with seven variants in *DNMT3A, TCN2, AS3MT, SLC19A1,* and *MTHFS* genes.

Our findings support previous results showing that females have significantly lower levels of LINE-1 methylation (El-Maarri *et al.* 2007, Zhu *et al.* 2012, Wilhelm *et al.* 2010, El-Maarri *et al.* 2011). DNA methylation is important for X-chromosome inactivation in females (Jones & Liang 2009), and although LINE-1 sequences do not seem to be the major mechanism involved in this process, they may be involved in spreading the X-inactivation signal across the chromosome (Bailey et al. 2000). In support of this, a recent study showed that LINE-1 sequences were hypomethylated in the inactive X-chromosome (Singer et al. 2012). A small study of 33 men and 33 women reported lower levels of blood SAM in women (Poirier et al. 2001). Hormonal factors may also contribute to the difference in methylation levels between genders. However, a recent *in vitro* study assessing the role of estrogen, progesterone and dihydrotestosterone on DNA methylation in four cell lines found no detectable effect of these hormones on methylation levels at the LINE-1 and *Alu* repeats (El-Maarri et al. 2011). Further studies are needed to decipher the relationship between gender and LINE-1 methylation.

Because tobacco smoking is an important contributor to disease and is a modifiable behavioral factor, there has been much interest in the relationship between smoking and DNA methylation. Our findings are in line with other studies that reported no association between LINE-1 methylation and smoking status (Terry et al. 2011). In the present study, we found that subjects who smoked blond tobacco had lower levels of global DNA methylation than nonsmokers. An experimental study has shown that cigarette smoke condensates can induce DNA demethylation in repeat elements, such as LINE-1 and D4Z4 (Liu et al. 2010). Both black and blond tobacco cause disease although the former is more mutagenic, reflective of the higher levels of *N*-nitrosamines and aromatic amines in smoke produced by black tobacco (Malaveille et al. 1989). Our findings suggest that the toxic effects of blond tobacco could be mediated by

modulating the epigenetic landscape. This may have a public health implication given epigenetic alterations are reversible.

We also provide evidence that arsenic levels were inversely associated with LINE-1 methylation, and that arsenic may have a strong effect on LINE-1 methylation. For each μg/g increase in arsenic there was a 3.6% decrease in DNA methylation level. This inverse association is in agreement with that from a population-based study that used a similar assay to assess LINE-1 methylation levels and toenail concentrations of arsenic (Wilhelm et al. 2010), as well as with several other experimental studies (Ren *et al.* 2011, Reichard & Puga 2010). The mechanisms through which arsenic exposure influences DNA methylation are not fully understood. Studies in cell lines and mouse models exposed to arsenic for up to 22 and 48 weeks, respectively have shown that prolonged exposure to sodium arsenite resulted in decreased global DNA methylation, and inhibition of *DNMT1, DNMT3A,* and *DNMT3B* gene expression (Ren *et al.* 2011, Reichard & Puga 2010). It is likely that through the combined effect of depleting the cellular pool of SAM and inhibiting the activity of *DNMTs*, both inorganic and organic arsenic may lead to decreased global DNA methylation.

We are not aware of any human studies associating iron and nickel levels and global DNA methylation. In the present study, iron and nickel showed a small but significant positive association with LINE-1 methylation level. Genes involved in hepatocellular carcinoma (HCC) have been found to be hypermethylated in hereditary hemochromatosis, a disease characterized by chronic iron overload that is a risk factor for HCC (Lehmann et al. 2007). Iron, together with 2-oxoglutarate and oxygen, is an essential cofactor for the ten-eleven translocation (*TET*) family of proteins that hydroxylate 5-methylcytosine to 5-hydroxymethylcytosine and further oxidize to 5-carboxylcytosine and 5-formylcytosine, which have all been suggested to be precursors for both active and passive DNA demethylation (Bhutani et al. 2011). Experimental studies conducted in Chinese hamster cell lines (G12) treated with nickel chloride for up to 3 weeks have shown that nickel chloride leads to both promoter hypermethylation and elevated total genomic DNA methylation (Lee *et al.* 1998, Lee *et al.* 1995). How nickel induces DNA methylation is not yet understood, but it has been proposed that nickel first induces chromatin condensation followed by *de novo* methylation of heterochromatic DNA (Lee et al. 1995).

The three SNPs with the strongest associations with LINE-1 methylation were all in *TCN2*, including two exonic SNPs that result in missense substitutions (rs9606756 and

rs9621049) and one intronic SNP (rs4820887). SNPs rs9621049 and rs4820887 have a linkage disequilibrium r^2 value of 0.8 implying that the observed effect in LINE-1 methylation may eventually be attributed to either of them. *TCN2* encodes for transcobalamin II which binds and transports vitamin B_{12} into the cell (Regec et al. 1995), which suggests that variations in *TCN2* could potentially impair the one-carbon metabolism pathway by altering the cytoplasmic concentration of vitamin B_{12}. *TCN2*-rs9606756 leads to an I23V substitution located at a NAGNAG tandem acceptor site that is a target of alternative splicing (Hiller et al. 2006). *TCN2*-rs9621049 leads to a S348F and may also play a role in the availability of vitamin B_{12} in the cell thereby affecting LINE-1 methylation levels.

Four SNPs in other genes (*DNMT3A*-rs7581217; *AS3MT*-rs7085104; *MTHFS*-rs1380642; *SLC19A1*-rs914238) involved in the one-carbon metabolism were also associated with global DNA methylation in our study population. *DNMT3A*, a *de novo* DNA methyltransferase, establishes the patterns of methylation in early embryonic development, along with *DNMT3B*, and cooperates with *DNMT1* to maintain the methylation of repetitive sequences, such as LINE-1 and *Alu* elements (Jones & Liang 2009). Recurrent mutations in *DNMT3A* have been associated with adult hematologic malignancies (Ley *et al.* 2010, Yan *et al.* 2011), and mice lacking *Dnmt3a* die in the first weeks of postnatal life (Robertson 2005). The product of *AS3MT* catalyzes the conversion of trivalent arsenic by addition of a methyl group to monomethylarsonic acid and dimethylarsonic acid (Ren et al. 2011); monomethylarsonic acid being the most toxic metabolite (Engstrom et al. 2011). rs7085104, located in the promoter region of *AS3MT*, has been associated with arsenic metabolism, as evidenced by differences in urinary concentration of arsenic metabolites (Engstrom *et al.* 2011, Valenzuela *et al.* 2009). We also observed a significant interaction of this SNP with levels of arsenic on LINE-1 methylation levels. While subjects with at least one copy of the major allele had a 4.1% decrease in methylation level for 1-μg/g increase in arsenic, which is comparable to the overall population (-3.6%), those homozygous for the variant allele had a 10% increase in LINE-1 methylation. These findings support the putative functionality of the association. The product of *MTHFS* catalyzes the conversion of 5-formyltetrahydrofolate to 5,10-methenyltetrahydrofolate and a genome-wide association study reported an association between a variant in this gene and chronic kidney disease (Kottgen et al. 2008). *SLC19A1* is a ubiquitously expressed major transporter of folate and antifolates and regulator of the intracellular concentrations of folate (Matherly et al. 2007).

Common variants in this gene have been associated with plasma folate levels, various types of cancer (esophageal, gastric and acute lymphoblastic leukemia), and altered methotrexate transport and adverse effects of methotrexate (Matherly et al. 2007).

Among the limitations of the study is the majority of individuals were of advanced age (mean age 64 years, SD=10 years) and men. This may explain the lack of association between DNA methylation and age in our study population, in contrast with other studies that included subjects with a broader age range (Fraga *et al.* 2005a). Thus, our findings refer to an adult population of mostly men. Results were consistent with estimates for the population as a whole when stratified by gender, with the exception of nickel, tobacco type, and *MTHFS*-rs1380642 which become nonsignificant while the point estimates were in the same direction (data not shown). These differences may reflect reduced power to estimate associations among women due to the small sample size. The presence of missing data for some of the variables might have resulted in decreased power but even with the available sample size we were able to reproduce previous results and identify novel predictors of LINE-1 methylation. Furthermore, while the study subjects were recruited from hospitals, none of reasons for hospitalization were significantly associated with LINE-1 methylation.

Strengths of the study include its size, the availability and quality of individual data on demographics, lifestyle, environmental exposures, and genetics. Additionally, we assessed LINE-1 methylation levels, which are considered a good marker of global DNA methylation (Yang et al. 2004), using pyrosequencing, which gives accurate and reproducible measurements (Laird 2010, Estecio *et al.* 2007, Tost & Gut 2007). Furthermore, this assessment was made using DNA from granulocytes, avoiding a possible effect of cell blood count in our study.

To the best of our knowledge, this is the first study to identify seven SNPs in association to changes in LINE-1 methylation and to integrate different types of information to assess the determinants of global methylation in blood DNA. Integration of both internal and external exposure data in this study is a step forward in understanding how the exposome modulates DNA methylation patterns.

In conclusion, the current study provides further evidence that DNA methylation levels are influenced by variants in genes involved in the one-carbon metabolism pathway, and exposure to trace elements and tobacco smoke. Given the fact that smoking and some of the genetic variants and trace elements associated with LINE-1 methylation in the present study have

also been associated with adverse health outcomes including cancer, our results provide additional insight into the potential mechanism through which these agents participate in the development of those diseases. Furthermore, these factors should be considered as potential confounders in etiologic and interventional studies analyzing the role of DNA methylation in disease. Nevertheless, future studies are required to replicate and extend our findings in different populations.

CHAPTER IV. LINE-1 METHYLATION IN LEUKOCYTE DNA, INTERACTION WITH PHOSPHATIDYLETHANOLAMINE N-METHYLTRANSFERASE VARIANTS, AND RISK OF UROTHELIAL CARCINOMA OF THE BLADDER

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Submitted

Abstract

Aberrant global DNA methylation is shown to increase cancer risk. LINE-1 has been proven a measure of global DNA methylation. The objectives of this study were to assess the association between LINE-1 methylation level and risk of urothelial carcinoma of the bladder (UCB), and to further explore effect modification by environmental and genetic factors. Bisulfite-treated leukocyte DNA from 952 cases and 892 controls of the Spanish Bladder Cancer/EPICURO Study was used to measure LINE-1 methylation level at four randomly selected CpG sites by pyrosequencing. Logistic regression models, adjusted for age, gender, region, and smoking, were fitted to estimate odds ratios (ORs) and 95% confidence intervals (95%CIs). Interactions between LINE-1 methylation level and environmental and genetic factors were assessed. The risk of UCB followed a non-linear association with LINE-1 methylation. Compared to subjects in the middle tertile, the adjusted OR for subjects in the lower and the higher tertiles were 1.26 (95%CI 0.99-1.60, P = 0.06) and 1.33 (95%CI 1.05-1.69, P = 0.02), respectively. This association significantly increased among individuals homozygous for the major allele of five single nucleotide polymorphisms located in the phosphatidylethanolamine *N*-methyltransferase (*PEMT*) gene (corrected P-interaction < 0.05). This is the first large-scale study showing that both low and high levels of global DNA methylation levels are associated with risk of UCB. Moreover, variants in *PEMT* seemed to markedly modify this risk. Our results may provide additional insights into the etiological mechanisms of DNA methylation in UCB development, and therefore deserve further exploration in independent populations.

Introduction

Urothelial carcinoma of the bladder (UCB) is one of the most common cancers in developed countries mainly affecting men (Parkin 2008). In Spain, the incidence rate of UCB has been increasing in the past three decades and the ratio of men to women developing the disease is 7:1 (Izarzugaza *et al.* 2010). Recurrence is a major problem in the management of UCB necessitating frequent follow-ups thus, making it one of the most expensive cancer types to treat (Lotan *et al.* 2008). Established risk factors for UCB include tobacco smoking, occupational exposures to aromatic amines, arsenic in drinking water (Murta-Nascimento *et al.* 2007, Volanis *et al.* 2010), and genetic variants of *NAT2* and *GSTM1*; the latter two have been shown to significantly modify the risk conferred by tobacco smoking (Garcia-Closas *et al.* 2005, Rothman *et al.* 2010). Recently, additional common genetic variants on *CBX6-APOBEC3A, CCNE1, MYC, PSCA, SLC14A1, TERT-CLPTM1L, TMEM129-TACC-FGFR3, TP63* and *UGT1A6* cluster have been associated with risk of UCB (Rothman *et al.* 2010, Garcia-Closas *et al.* 2011, Tang *et al.* 2012).

Genome-wide altered epigenetic states are common findings in several malignancies including bladder tumors. These epigenetic states include global DNA hypomethylation and gene promoter methylation, post-translational histone modifications and deregulation of microRNAs (Esteller 2008, Taby & Issa 2010). Global DNA hypomethylation in cancer is mostly found in repetitive sequences of the genome such as long interspersed nuclear element 1 (LINE-1) and *Alu* elements (Esteller 2008). It has been suggested that methylation represses the transcription of these repetitive regions to maintain genomic stability, and prevent mutations, deletions and insertions (Esteller 2008). In UCB, genomic instability has been observed at different stages of the disease (Florl & Schulz 2008). There is evidence that LINE-1 hypomethylation could also induce the expression of the *MET* oncogene in UCB and normal tissues (Wolff *et al.* 2010). Global DNA methylation measured in peripheral blood cells has been used to investigate its relationship with cancer risk, although results have been inconclusive (Woo & Kim 2012). Studies investigating the role of genomic DNA methylation (measured by direct quantification of 5-methylcytosine as well as at CpG sites in LINE-1 sequences) and UCB have inconclusively shown an inverse association between genomic DNA methylation and risk of UCB (Moore *et al.* 2008, Wilhelm *et al.* 2010, Cash *et al.* 2012). Whether environmental and genetic factors modify

the association between global DNA methylation and UCB risk is not known, although it has been suggested that smoking, B vitamins, arsenic, and genes involved in the one-carbon metabolism pathway may play a modifier role (Volanis *et al.* 2010, Moore *et al.* 2007). Hence, understanding the role of global DNA methylation in UCB development and its interaction with other known and potential risk factors may provide further insight on the etiology and susceptibility of this tumor.

The objectives of this study were to assess the risk of UCB associated with LINE-1 methylation levels, as a measure of global DNA methylation in peripheral blood, as well as to explore whether personal, lifestyle, environmental and genetic factors modify this association.

Methods

Study population: The Spanish Bladder Cancer/EPICURO Study is a multi-center, hospital-based case-control study conducted in five regions of Spain (Barcelona, Valles, Elche, Tenerife, and Asturias) between 1998 and 2001. The design of the study has been previously described elsewhere in detail (Garcia-Closas *et al.* 2005, Samanic *et al.* 2006). Briefly, cases and controls were recruited to the study from 18 hospitals. Cases were newly diagnosed subjects with histologically confirmed UCB. Controls were patients admitted to the same hospital as cases for reasons not related to exposures of interest and individually matched for age (± five years), gender and geographic region to cases. From the initial study population of 1219 cases and 1271 controls, 1107 cases and 1032 controls provided blood sample (before treatment was instituted). Of these, 995 cases and 925 controls, all Caucasians and without missing demographic data, had enough leukocyte DNA for sodium bisulfite treatment and subsequent measurement of DNA methylation. Because in 43 cases and 33 controls, quantification of DNA methylation failed, the final study population for the current analysis was 952 cases and 892 controls aged from 20 to 81 years. Written informed consent was obtained from each study subject before interview, and the study was approved by the US National Cancer Institute and local institutional review boards. Data on demographic and personal characteristics, smoking and other known and potential risk factors for UCB were collected by means of a computer assisted personal interview. Food frequency questionnaires were used to collect data on fruit, vegetable, B vitamins, and protein and alcohol intake over the five years before interview (Garcia-Closas *et al.* 2007b).

Analysis of LINE-1 methylation: Bisulfite modification of genomic DNA extracted from leukocytes using standard techniques was performed using EZ-96 DNA METHYLATION-GOLD™ KIT (Zymo Research, Irvin, CA, USA) following the recommendations of the manufacturer. PCR amplification of bisulfite-modified DNA was carried out using a set of forward and reverse primers reported previously (Estecio *et al.* 2007). To quantify the methylation level of each of the four CpG sites immediately after the sequencing primer, sequencing of the PCR product was performed by pyrosequencing using PyroMark™ Q24 System (QIAGEN, Valencia, CA, USA) as recommended by the manufacturer. Methylation level at each CpG site was extracted using PyroMark™ Application Software version 2.0.6 (QIAGEN, Valencia, CA, USA) and the value was expressed as the percentage of methylated cytosines over the sum of methylated and unmethylated cytosines. The average methylation level of the four LINE-1 CpG sites was used in the analysis as a marker of the global DNA methylation level. As a quality control measure, 307 randomly selected samples from cases and controls were run in duplicate and the within-sample coefficient of variation was 4.53%. The mean methylation level of the duplicates was used in the analysis.

Genotyping: Genotyping was carried out using DNA isolated form leukocytes as described previously (Garcia-Closas *et al.* 2005). All genotype assays used in this analysis were done at the Core Genotyping Facility of the Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, MD, USA. Methods of the SNP genotyping process were described previously (Garcia-Closas *et al.* 2005, Rothman *et al.* 2010, Garcia-Closas *et al.* 2007a). SNP genotyping was done using Illumina Infinium® Human1M-Duo (480 SNPs), Illumina GoldenGate® (36 SNPs) and TaqMan® (8 SNPs) assays. All genotypes included in the study were in Hardy-Weinberg equilibrium in the control population ($P > 0.05$). A total of 524 SNPs and copy number variation in *GSTM1* and *GSTT1* were assessed in the present study. Of these SNPs, 515 SNPs were located in 24 candidate genes involved in one-carbon metabolism pathway, including DNA methylation and arsenic metabolism (see Supplementary Table S4-1). These pathways are known to influence DNA methylation by altering the cellular concentration of the methyl group donor *S*-adenosylmethionine (SAM) (Ulrey *et al.* 2005, Lee *et al.* 2009, Moore *et al.* 2007). Additionally, eight SNPs identified in a recent multi-stage genome-wide association study of UCB, and a SNP rs1495741 tagging *NAT2* acetylator phenotype were also

included in the analysis (Rothman *et al.* 2010). SNPs and variants were investigated for their modifier effect on the association between LINE-1 methylation and UCB risk.

Quantification of trace elements: Methods of quantification of toenail trace elements level included in the current study have been previously described (Amaral *et al.* 2011). Briefly, after cleaning and washing the toenails to remove external contaminants, trace elements were quantified at the Trace Element Analysis Core (Dartmouth College, NH, USA), using inductively coupled plasma-mass spectrometry (Hopkins *et al.* 2004). Toenails were digested with Optima nitric acid (Fisher Scientific, St. Louis, MO), at 105 °C, then hydrogen peroxide and further heating the dilution with deionized water. All sample preparation steps were recorded gravimetrically. For quality control, each batch of analyses accommodated six standard reference material samples with known trace element content (SRM; GBW 07601, powdered human hair) and six analytic blanks, together with the study samples. Overall, concentrations (μg/g) of 12 trace elements (aluminum, arsenic, cadmium, chromium, copper, iron, lead, manganese, nickel, selenium, vanadium, and zinc) were determined.

Statistical methods: Pearson chi-square test was used to evaluate the association between case-control status and categorical variables. Normality of LINE-1 methylation distribution was assessed graphically by plotting histograms and formally tested by using Shapiro-Wilk test for normality. The non-parametric Mann-Whitney *U* test was used to compare medians of continuous variables in different strata.

To estimate the risk associated with global DNA methylation, LINE-1 methylation level was modeled using restricted cubic splines in a logistic regression model adjusting for age (continuous), gender, region, and smoking status (never, occasional, former and current smoker). Upon the visualization of a non-linear "U-shape" risk pattern from the cubic spline regression (Supplementary Figure 4-1), LINE-1 methylation levels were categorized into three categories by using tertile cut-points (56.68% and 58.65%) based on its distribution among the control population. For subsequent analyses, this variable was used by assigning the middle tertile as the referent category. Unadjusted and adjusted logistic regression models were fitted to estimate odds ratios (ORs) and the corresponding 95% confidence intervals (95%CI). In addition to LINE-1 methylation, variables included in the basic multivariable adjusted model were age, gender, region and smoking status. Association between LINE-1 methylation, in tertiles, at each of the

four LINE-1 CpG sites and risk of UCB was evaluated. Also, and in order to mimic the distribution of LINE-1 methylation levels in tertiles using a continuous variable, the association between the absolute deviation (continuous) from the median LINE-1 methylation level was modeled by logistic regression unadjusted and adjusted for age, gender, region, and smoking status. Moreover, the association between LINE-1 methylation and UCB subphenotypes (low-, high-grade non-muscle-invasive bladder cancer (NMIBC) and muscle-invasive bladder cancer (MIBC)) was modeled using multinomial logistic regression. Likelihood ratio test for heterogeneity was used to test whether the ORs for low-, high-grade NMIBC and MIBC are different from each other.

Interaction between LINE-1 methylation and individual covariates [age (<60, 60-69, and ≥ 70 years), gender, smoking status (never and ever smoker), diet factors, trace elements (continuous) and SNPs (four modes of inheritance)] was tested by including a multiplicative interaction term into the logistic regression model. For the interaction between LINE-1 methylation and smoking, additional analyses included adjustment among regular smokers for each of the following, as a continuous variable: pack-years, duration of cigarette smoking, and number of cigarettes smoked per day. Significance of the interaction term was tested by comparing the models with and without the interaction term using the likelihood ratio test. A permutation test done by randomly sampling 10,000 times and assigning case/control status in a proportion similar to the current study was applied to estimate interaction *P* for each SNP, corrected for multiple testing. All statistical tests were two-sided, and a *P* less than 0.05 was considered significant. Statistical analyses were carried out by using STATA/SE, version 10.1 (StataCorp, College Station, Texas, USA).

Results

The characteristics of cases and controls are shown in Table 4-1. Subjects were mostly men and smokers. A large proportion of cases (78%) were diagnosed with a NMIBC. The distribution of global DNA methylation was bimodal. The median (interquartile range) LINE-1 methylation levels among cases and controls were 57.60% (4.06%) and 57.44% (3.36%), respectively (P = 0.3). The minimum and maximum values of LINE-1 methylation were 28.81% and 85.44% among cases and 37.91% and 85.68% among controls.

Compared to subjects in the middle tertile of LINE-1 methylation, the adjusted ORs were 1.26 (95% CI 0.99-1.60, P = 0.06) and 1.33 (95% CI 1.05-1.69, P = 0.02) for the lowest and highest tertiles, respectively. These ORs were very similar to the crude ones (Table 4-2). The associations between methylation level at each of the four CpG positions of LINE-1 and risk of UCB were similar to the results using the average of LINE-1 methylation level (Supplementary Table S4-2). The association for the absolute deviation from the median LINE-1 methylation level, modeled as a continuous variable and adjusted for age, gender, region and smoking status, was similarly significant (adjusted OR = 1.03, 95%CI 1.01-1.05, P = 0.008). When further analysis was carried out separately for low-, high-grade NMIBC and MIBC, ORs for LINE-1 methylation levels were similar to the overall OR and no heterogeneity was found between the three UCB subphenotypes (P > 0.05) (Supplementary Table S4-3).

Table 4-1 Distribution of characteristics of cases and controls with LINE-1 methylation data in the SBC/EPICURO study

Variables	Cases, n (%), N=952	Controls, n (%), N=892	P-value
Age in years, median (IQR)	68 (13)	66 (13)	<0.00001*
Gender			
Male	826 (86.8)	792 (88.8)	0.2
Female	126 (13.2)	100 (11.2)	
Region			
Barcelona	162 (17.0)	168 (18.8)	0.7
Valles	148 (15.5)	135 (15.1)	
Elche	72 (7.6)	73 (8.2)	
Tenerife	173 (18.2)	145 (16.3)	
Asturias	397 (41.7)	371 (41.6)	
Smoking status			
Never	137 (14.5)	255 (28.7)	<0.00001
Occasional	34 (3.6)	66 (7.4)	
Former	376 (39.7)	329 (37.0)	
Current	399 (42.2)	239 (26.9)	
Missing	6	3	
Sub-phenotypes			
Non-muscle invasive	708 (77.6)	—	
Muscle invasive	204 (22.4)	—	
Missing	40	—	

*Mann–Whitney *U* test p-value.
IQR, interquartile range.

Table 4-2 Association between LINE-1 methylation and urothelial carcinoma of the bladder risk in the SBC/EPICURO study

	Cases	Controls	Crude OR	95% CI		P-value
LINE-1 methylation (tertiles)						
T1 (< 56.68%)	337	298	1.29	1.02	1.62	0.03
T2 (56.68 -< 58.65%)	261	297	1 Referent			
T3 (≥ 58.65%)	354	297	1.36	1.08	1.70	0.008

	Cases	Controls	OR*	95% CI		P-value
LINE-1 methylation (tertiles)						
T1 (< 56.68%)	335	296	1.26	0.99	1.60	0.06
T2 (56.68 -< 58.65%)	260	297	1 Referent			
T3 (≥ 58.65%)	351	296	1.33	1.05	1.69	0.02

*Adjusted for age, gender, region (Barcelona, Valles, Elche, Tenerife and Asturias) and smoking status (never, occasional, former and current smoker)

The effect of LINE-1 on UCB risk was not modified by age, gender, tobacco type, nutrient intakes, or trace elements (Supplementary Tables S4-4 and S4-5). Although the risk of UCB associated with low and high levels of LINE-1 methylation was significant among never smokers, the interaction between LINE-1 methylation and smoking status was not significant (*P* = 0.2; Supplementary Table S4-4). This interaction was not significant even when comparing never smokers with regular smokers, after adjusting the models for duration of cigarette smoking, cigarettes smoked per day or pack-years among smokers (data not shown). There was no interaction between variation in *GSTM1* or *GSTT1* or SNPs previously associated with UCB risk and LINE-1 methylation levels. The same is true for most of the SNPs in genes from the one-carbon metabolism pathway (Supplementary Tables S4-5 and S4-6). However, the increased risk of UCB among subjects in the lowest and highest tertiles was significantly modified by five SNPs (rs2124344, rs7215833, rs4646340, rs4646350, and rs4646341) in the phosphatidylethanolamine *N*-methyltransferase (*PEMT*) gene. Under the dominant mode of inheritance, the risk of UCB being in the lowest or highest tertile of LINE-1 methylation was significantly increased only among individuals homozygous for the major allele (*P*-interaction $\leq 4.9 \times 10^{-5}$; *P*-interaction ≤ 0.03 corrected for multiple testing; Table 4-3).

Table 4-3 Interaction between LINE-1 methylation and single nucleotide polymorphisms in phosphatidylethanolamine N-methyltransferase (PEMT) in the SBC/EPICURO study

rs2124344[CC], *PEMT*, intron

LINE-1 methylation (tertiles)	Cases	Controls	OR	95% CI		P-value
T1 (<56.68%)	148	112	2.26	1.52	3.36	$5.2x10^{-5}$
T2 (56.68-<58.65%)	76	132	1 (Ref.)			
T3 (≥58.65%)	158	107	2.69	1.80	4.00	$1.2x10^{-6}$

rs2124344 [CT-TT], *PEMT*, intron

LINE-1 methylation (tertiles)	Cases	Controls	OR	95% CI		P-value	P-value interaction*	Corrected P-value interaction†
T1 (<56.68%)	179	177	0.89	0.65	1.21	0.4	$1.5x10^{-5}$	0.01
T2 (56.68-<58.65%)	181	160	1 (Ref.)					
T3 (≥58.65%)	184	183	0.86	0.63	1.18	0.4		

rs7215833[CC], *PEMT*, flanking 5'UTR

LINE-1 methylation (tertiles)	Cases	Controls	OR	95% CI		P-value
T1 (<56.68%)	155	119	2.13	1.44	3.14	0.0002
T2 (56.68-<58.65%)	77	131	1 (Ref.)			
T3 (≥58.65%)	155	101	2.62	1.76	3.91	$2.4x10^{-6}$

rs7215833[CT-TT], *PEMT*, flanking 5'UTR

LINE-1 methylation (tertiles)	Cases	Controls	OR	95% CI		P-value	P-value interaction*	Corrected P-value interaction†
T1 (<56.68%)	172	171	0.89	0.65	1.23	0.5	$3.6x10^{-5}$	0.02
T2 (56.68-<58.65%)	180	161	1 (Ref.)					
T3 (≥58.65%)	187	189	0.87	0.64	1.19	0.4		

rs4646340[AA], *PEMT*, intron‡

LINE-1 methylation (tertiles)	Cases	Controls	OR	95% CI		P-value
T1 (<56.68%)	153	116	2.13	1.43	3.16	0.0002
T2 (56.68-<58.65%)	75	126	1 (Ref.)			
T3 (≥58.65%)	156	99	2.69	1.79	4.04	$1.8x10^{-6}$

rs4646340 [AG-GG], *PEMT*, intron

LINE-1 methylation (tertiles)	Cases	Controls	OR	95% CI		P-value	P-value interaction*	Corrected P-value interaction†
T1 (<56.68%)	174	174	0.92	0.67	1.25	0.6	$3.8x10^{-5}$	0.02
T2 (56.68-<58.65%)	182	166	1 (Ref.)					
T3 (≥58.65%)	186	191	0.88	0.65	1.19	0.4		

rs4646350[CC], *PEMT*, intron

LINE-1 methylation (tertiles)	Cases	Controls	OR	95% CI		P-value
T1 (<56.68%)	148	115	2.16	1.45	3.20	0.0001
T2 (56.68-<58.65%)	76	130	1 (Ref.)			
T3 (≥58.65%)	156	106	2.64	1.77	3.94	$2.1x10^{-6}$

rs4646350[CT-TT], *PEMT*, intron

LINE-1 methylation (tertiles)	Cases	Controls	OR	95% CI		P-value	P-value interaction*	Corrected P-value interaction†
T1 (<56.68%)	179	175	0.91	0.66	1.24	0.5	$4.9x10^{-5}$	0.03
T2 (56.68-<58.65%)	181	162	1 (Ref.)					
T3 (≥58.65%)	186	184	0.88	0.65	1.20	0.4		

All models are adjusted for age, gender, region and smoking status (never, occasional, former and current smoker).

*Uncorrected likelihood ratio test p-value.

†Likelihood ratio test p-value corrected for multiple testing using permutation test.

‡rs4646341 was in perfect LD with rs4646340 (r2=1.0, D'=1.0) and the corrected p-value for interaction of this SNP was 0.037.

MAF - rs2124344=0.36, rs7215833=0.36, rs4646340=0.37, rs4646350=0.36 and rs46341=0.37.

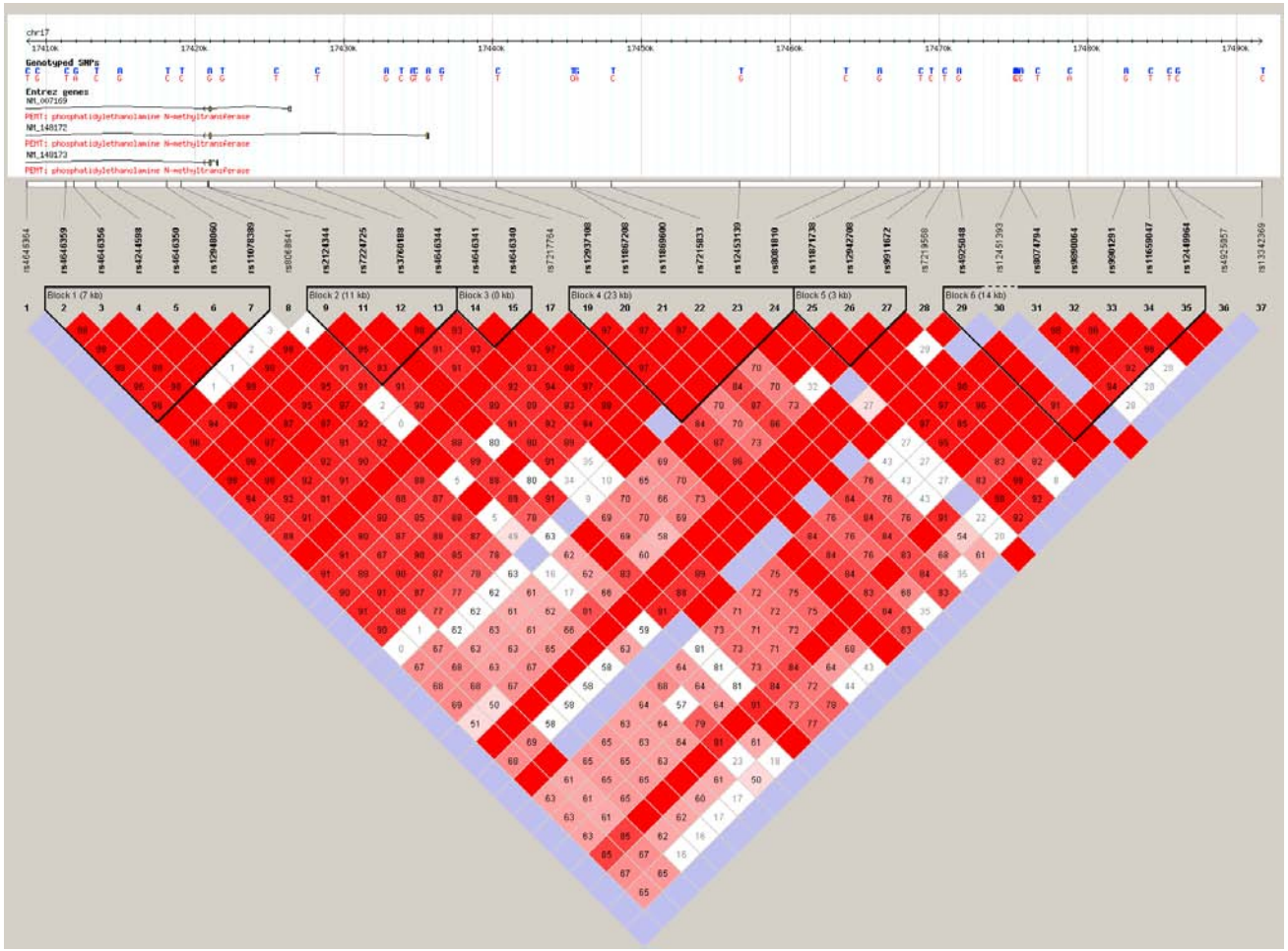

Figure 4-1 Linkage disequilibrium plot for SNPs in phosphatidylethanolamine N-methyltransferase (*PEMT*) including the five SNPs analyzed in the current study

Discussion and conclusions

The current study shows that both low and high levels of LINE-1 methylation are associated with increased risk of UCB. This association was significantly modified by five SNPs in the *PEMT* gene. The results show, for the first time, a non-linear - "U-shaped" - association between global genomic DNA methylation level and UCB risk, which was elevated among subjects having specific genotypes of *PEMT*. Altogether, points to the complex etiological mechanisms of DNA methylation in UCB development.

The finding of increased risk of UCB among individuals with low level of methylation is in agreement with previous reports (Moore *et al.* 2008, Wilhelm *et al.* 2010, Cash *et al.* 2012). Moore *et al.*, by analyzing global 5-methylcytosine content of the genome using *HpaII*, high-performance capillary electrophoresis and densitometry, showed that low level of global DNA methylation was associated with increased risk of UCB in a subsample of the same study (Moore *et al.* 2008). Additionally, two studies also using sodium bisulfite modification of leukocyte DNA, followed by pyrosequencing to quantify methylation levels of four LINE-1 CpG sites reported a similar inverse association between LINE-1 methylation level and risk of UCB in independent populations (Wilhelm *et al.* 2010, Cash *et al.* 2012). An experimental study demonstrated hypomethylation of LINE-1 sequences in urothelial carcinoma cell lines and tumors compared to normal bladder tissue (Jurgens *et al.* 1996). Another study also showed that hypomethylation of LINE-1 promoter can induce the expression of the *MET* oncogene in bladder tumors and normal urothelial tissues (Wolff *et al.* 2010). Interestingly, the current study also shows that higher methylation level was associated with increased risk of UCB. In support of this finding, a recent case-control study that investigated the association between LINE-1 methylation and renal cell carcinoma showed a direct association between methylation and risk of renal cell carcinoma (Liao *et al.* 2011), while another study that evaluated LINE-1 methylation status in normal and cancerous cells found out sporadic hypermethylation of LINE-1 loci in cancer cells (Phokaew *et al.* 2008). Recent studies have demonstrated that methylation blocks the initiation of transcription of repetitive DNA sequences such as LINE-1 and *Alu* elements, while at the same time allowing transcriptional elongation of the host gene suggesting that methylation at repetitive sequences might have dual functions in addition to silencing repetitive sequences depending on the location and context of these regions (Jones 2012).

To our knowledge, the present study is the first to show a significant interaction between LINE-1 methylation and SNPs in *PEMT* gene on the risk of UCB. There is no available data on UCB risk and *PEMT* polymorphisms. However, a population-based study identified a SNP in a promoter region of *PEMT* to be associated with increased risk of breast cancer (Xu *et al.* 2008). *PEMT* is located within the Smith-Magenis syndrome region on chromosome 17, and encodes for a transmembrane protein involved in important cellular processes including choline and lipid metabolism, insulin sensitivity and homocysteine levels (Vance *et al.* 2007). *PEMT* methylates, using SAM as a substrate, phosphatidylethanolamine to phosphatidylcholine, which accounts for 95% of total choline pool in tissues (Ueland 2011, Vance *et al.* 2007). Evidence suggest that phosphatidylcholine metabolism could be involved in malignant transformations (Glunde *et al.* 2011). Experimental studies have demonstrated altered choline metabolism in tumors, mediated by transcription factors (signaling pathways) associated with oncogenesis such as hypoxia-inducible factors 1, and by the *RAS* and *PI3K/AKT* pathways (Glunde *et al.* 2011). It has been known that these pathways are constitutively activated in the majority of bladder tumors, namely low-grade non-muscle-invasive papillary tumors, which account for 70-80% of UCB cases (Wu 2005, Lopez-Knowles *et al.* 2006). On the other hand, upon oxidation choline gives rise to betaine which promotes remethylation of homocysteine to methionine affecting the concentration of SAM (Ueland 2011). Therefore, polymorphisms in *PEMT* could directly affect the one-carbon metabolism pathway and impair DNA methylation. The five SNPs are located in flanking 5'UTR and intron of *PEMT* (Figure 4-1). Alternatively, we cannot rule out these associations could be due to chance.

Unlike the previous studies where linear associations were observed between LINE-1 methylation and cancer risk, in this study increased risk of UCB at both extremes of LINE-1 methylation was observed. The risk pattern differences with the other studies could be explained by the larger sample size in the present that enables to further characterize the risk patterns as well as differences in methods used to quantify DNA methylation. A recent meta-analysis of LINE-1 methylation and UCB risk found a significant heterogeneity between assays used to quantify methylation (Woo & Kim 2012). That same analysis also identified significant heterogeneity among studies. It should be noted that LINE-1 methylation level in the current study population (mean = 59.2%) were substantially different from those in the previous two studies on UCB risk and LINE-1 methylation that reported mean methylation level of 79.6%

(Wilhelm *et al.* 2010), and 81.9% (Cash *et al.* 2012). This difference is not surprising and probably results from the fact that distinct CpG sites were analyzed. On the other hand, the differences could perhaps be explained by geographic dissimilarities resulting in differential exposure to environmental factors, including diet, or genetic background and stochastic factors present in these populations.

The limitations of this study include the fact that blood samples were collected at the time of interviews, before initiation of treatment. Some individuals were not included in the present analyses due to the lack of LINE-1 methylation data, however there was no relevant difference in selected characteristics except age (among controls) and gender (among cases and controls) between those with and without that data (Supplementary Table S4-7). To minimize the potential bias, all the models were adjusted for age, gender as well as region and smoking status. DNA methylation is tissue specific and it could be affected by cell type. To evaluate if there is a difference in methylation level among blood cell types, LINE-1 methylation level was quantified in DNA separately isolated from granulocytes and lymphocytes, the most abundant white blood cell types. There was no difference in LINE-1 methylation level between these cell types, which is in agreement with studies that found significant correlations between LINE-1 methylation assays of different blood cell types among healthy individuals (Wu *et al.* 2011a) as well as UCB patients (van Bemmel *et al.* 2012). However, the study on UCB patients also showed lack of correlation between global DNA methylation in blood and bladder tissue (van Bemmel *et al.* 2012), while another study showed LINE-1 methylation to be consistent from tissue to tissue indicating that tissue heterogeneity might not be associated with LINE-1 methylation levels (Choi *et al.* 2009).

The study has also important strengths. First is sample size: to the best of our knowledge this is the first large study to investigate the association between LINE-1 methylation and UCB risk (and any other cancer type). Second, the study integrates a wide range of information, including epidemiologic, clinical, environmental, and genetic data. Third, the method used to quantify LINE-1 methylation has been shown to be a good surrogate marker of global DNA methylation (Yang *et al.* 2004). Determining DNA methylation levels by pyrosequencing provides reproducible and accurate results (Tost & Gut 2007, Laird 2010).

In conclusion, this study showed that both low and high levels of global DNA methylation may be associated with increased risk of UCB, and that this risk can be markedly modified by *PEMT*. Taken together, the results suggest that DNA methylation and the one-carbon metabolism pathway play a relevant role in the development of UCB. Global DNA methylation may have a more complex association with UCB than previously thought. In addition, the findings suggest that DNA methylation measured in peripheral blood cells could be further explored to be used as a susceptibility marker for UCB risk. Nevertheless, further studies in large and independent populations are required.

CHAPTER V. SUBTELOMERIC MACROSATELLITE REPEAT D4Z4 METHYLATION: GENETIC AND NON-GENETIC PREDICTORS AND RISK OF UROTHELIAL CARCINOMA OF THE BLADDER

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Abstract

Epigenetic alterations are a hallmark of carcinogenesis. While methylation of the subtelomeric macrosatellite repeat D4Z4 has been implicated in disease, little is known about its genetic and non-genetic predictors and whether it is associated with urothelial carcinoma of the bladder (UCB) risk. The objectives of this study were to identify the predictors of D4Z4 methylation and to assess the association between D4Z4 methylation and risk of UCB. Levels of D4Z4 methylation were measured by pyrosequencing in bisulfite-modified DNA from granulocytes of 707 cases and 718 controls included in the Spanish Bladder Cancer/EPICURO Study. Robust linear regression models including non-genetic and genetic characteristics of controls were fitted to identify predictors of D4Z4 methylation. The association between D4Z4 methylation and UCB risk was analyzed using multivariable adjusted logistic regression, and odds ratios (ORs) and 95% confidence intervals (CIs) were estimated. Interaction between D4Z4 methylation and both established and potential risk factors for UCB was tested by including interaction terms in the models. In analyses limited to the controls, being female (β = -3.3; 95%CI -6.0 to -0.6, p-value = 0.02) or smoker of blond tobacco (β = -3.7; 95%CI -6.5 to -0.9, p-value = 0.01) was associated with decreased levels of D4Z4 methylation. Selenium was strongly inversely associated with D4Z4 methylation (β for 1-μg/g increase = -8.1; 95%CI -12.7 to -3.5, p-value = 0.0006). Individuals with D4Z4 methylation levels above or equal to the median ($\geq$ 64.4) had a slightly increased risk of UCB (OR = 1.23; 95%CI 0.99 - 1.53, p-value = 0.06). This association was modified by iron (p-interaction = 0.003), manganese (p-interaction = 0.04), and zinc (p-interaction = 0.01). Additionally, carriers of the minor allele of *FOLH1*-rs11040387 had a 3.5-fold increased risk of UCB (95%CI 1.94 - 6.44, p-value = 5.1×10^{-5}, corrected p-interaction = 0.02). In an analysis by UCB subtypes, D4Z4 hypermethylation was significantly associated with increased risk of developing low-risk tumors with FGFR3 overexpression (OR = 1.63; 95%CI 1.08 - 2.47, p-value = 0.02), but not with the other subtypes ($p_{heterogeneity}$ = 0.01). This is the first study showing D4Z4 hypermethylation in granulocyte DNA to be predicted by non-genetic factors (gender, tobacco, and selenium) and to be associated with UCB risk, particularly with the predominant NMIBC, and effect modifications by trace elements and *FOLH1*. These findings underline the potential of D4Z4 methylation for the development of a novel biomarker for UCB.

Introduction

Urothelial carcinoma of the bladder (UCB), one of the most common cancers in the Western world, is a heterogeneous group of tumors and develops along two divergent pathways characterized by distinct clinical and molecular features. Non-muscle-invasive bladder cancer (NMIBC), the predominant type of UCB (75-85%), harbors activating mutations in *FGFR3*, *PIK3CA* and *RAS* oncogenes while muscle-invasive bladder cancer (MIBC) is characterized by inactivating mutations in *TP53* and *RB* genes (Cheng *et al.* 2011, Knowles 2008, Falke & Witjes 2011). NMIBC is further divided into low- and high-risk groups that have varying risk of tumor recurrence and progression (Donat 2003, Falke & Witjes 2011). High rates of recurrence, mainly in NMIBC, make UCB the most expensive cancer to manage per patient (Sievert *et al.* 2009). Risk factors for UCB include cigarette smoking, exposure to arsenic in drinking water and to specific occupational carcinogens, and genetic variations in 12 genes, among them *NAT2* slow acetylator phenotype, *GSTM1* null genotype (Murta-Nascimento *et al.* 2007, Garcia-Closas *et al.* 2005, Rothman *et al.* 2010).

Aberrant epigenetic events, such as DNA methylation and histone modifications, are hallmarks of cancer, and can also interact with genetic changes to drive initiation and progression of cancer (Baylin & Jones 2011). Alterations in global and gene-specific DNA methylation have been observed in UCB (Heyn & Esteller 2012, Esteller 2008). Studies focusing on global 5-methylcitosine and long interspersed nuclear element-1 (*LINE-1*) have reported an association between methylation in leukocyte DNA and risk of UCB (Moore *et al.* 2008, Wilhelm *et al.* 2010, Cash *et al.* 2012). Methylation in other DNA sequences, such as D4Z4, has been shown to be relevant for disease.

D4Z4 is a subtelomeric macrosatellite repeat array located on chromosomes 4q, 10q, and all acrocentric chromosomes (van der Maarel *et al.* 2012, Lyle *et al.* 1995). Each unit of this array contains a retrogene double homeobox 4 (*DUX4*), a transcription factor that has been shown to regulate genes that play a role in relevant cellular pathways (van der Maarel *et al.* 2012). D4Z4 repeats have extremely high GC content, compared to the rest of the genome (Ehrlich *et al.* 2007), and hypomethylation has been found associated with facioscapulohumeral muscular dystrophy (FSHD) (van der Maarel *et al.* 2012), immunodeficiency, centromeric region

instability, and facial anomalies (ICF) syndrome (Kondo *et al.* 2000). However, in cancer tissues conflicting results ranging from hypomethylation to hypermethylation have been described (Cadieux *et al.* 2006, Cheng *et al.* 2004, Choi *et al.* 2009, Fraga *et al.* 2005b, Katargin *et al.* 2009, Tsumagari *et al.* 2008, Martinez *et al.* 2012). One study showed D4Z4 hypermethylation in UCB tissues compared to normal and adjacent normal tissues (Choi *et al.* 2009).

The predictors of D4Z4 methylation are unknown, and no population study has systematically addressed the role of D4Z4 methylation in UCB and its interaction with environmental and genetic factors. Thus, the objectives of this study were to identify the predictors of D4Z4 methylation in granulocyte DNA, to assess the association between D4Z4 methylation and UCB risk, and to explore effect modifications by selected personal, lifestyle, nutritional, environmental and genetic factors.

Methods

Study population: Individuals involved in the present analysis come from the Spanish Bladder Cancer/EPICURO Study. This is a large case-control study of 1219 cases and 1271 controls conducted between 1998 and 2001 in five regions of Spain (Asturias, Barcelona, Elche, Tenerife, and Valles). Details on methods of the study have been described elsewhere (Garcia-Closas *et al.* 2005, Samanic *et al.* 2006). Cases were incident UCB between 20 and 81 years of age. Diagnosis for each patient was verified by a panel of expert pathologists according to the 1998 ISUP/WHO classification system (Epstein *et al.* 1998). Controls were patients admitted to the same hospital as in cases for reasons not associated with exposures of interest. Controls were individually matched for age (± 5 years), gender, and region to cases. A total of 736 cases and 740 controls had enough bisulfite-modified DNA for the quantification of D4Z4 methylation levels at six CpG sites. Of these, 23 cases and 22 controls were excluded because of missing values in at least one of the six CpG sites. Three cases were excluded because of missing data in age and gender. To limit heterogeneity, three non-white cases were also excluded from the analyses. For this analysis, the final study population was 707 cases and 718 controls. Using a computer-assisted personal interview, data on demographic and personal characteristics were collected. Data on B vitamins, fruit and vegetable, protein and alcohol intake were assessed using food frequency questionnaires (Garcia-Closas *et al.* 2007b). Written informed consent was obtained from each study participant before interview, and the study was approved by the US National Cancer Institute and local institutional review boards.

D4Z4 methylation quantification using pyrosequencing: DNA isolated from granulocytes by standard methods was treated with sodium bisulfite using EZ-96 DNA METHYLATION-GOLD™ KIT (Zymo Research, Irvin, CA, USA), following the manufacturer's protocol. PCR amplification of bisulfite-modified DNA was carried out using a set of forward primer (GGTGGTTYGGGGTAGGG), reverse primer (biotin-CCCAAAAAAAAATAACAATTCTC), and sequencing primer (GGGAATATTTGGTTGGTTA) reported previously (Martinez *et al.* 2012). The pyrosequencing reaction was performed using PyroMark™ Q24 System (QIAGEN, Valencia, CA, USA) according to the manufacturer's recommendations. The methylation level at six CpG sites next to the sequencing primer was measured and the results were calculated by PyroMark™

Application Software version 2.0.6 (QIAGEN, Valencia, CA, USA). Bisulfite treatment of DNA converted unmethylated cytosine to uracil, which becomes thymine upon pyrosequencing, while the methylated cytosine remains unchanged. The mean D4Z4 methylation level at each of the six CpG positions was expressed as a percentage of unconverted methylated cytosine (C) over the total number of unconverted cytosine and thymine (T)= (C/C+T)*100%. As a quality control measure, 224 samples (121 cases and 103 controls) were run in duplicates and the within sample coefficient of variation was 0.88%.

Genotyping: We selected 496 SNPs with minor allele frequency greater than or equal to 5% from 24 candidate genes (*ALDH1L1, AS3MT, ATIC, BHMT, CBS, CHDH, CTH, DHFR, DNMT1, DNMT3A, DNMT3B, FOLH1, GART, GNMT, MTHFD, MTHFR, MTHFS, MTR, MTRR, PEMT, SHMT1, SLC19A1, TCN2, TYMS*), which are known to play a role in the one-carbon metabolism pathway, DNA methylation and arsenic metabolism. Methods of genotyping have been previously described (Garcia-Closas *et al.* 2005, Garcia-Closas *et al.* 2007a, Rothman *et al.* 2010). All SNPs were in Hardy-Weinberg equilibrium (p-value > 0.05). Additionally, data on copy number variation in xenobiotic-metabolizing genes *GSTM1* [present (++/+-) and null (--) genotypes], *GSTT1* [present (++/+-) and null (--) genotypes], and *NAT2* acetylator phenotype (rapid/intermediate and slow) were also included in the analyses.

Trace elements analysis: Toenails were collected to measure the concentration of 12 trace elements (aluminum, arsenic, cadmium, chromium, copper, iron, lead, manganese, nickel, selenium, vanadium, and zinc). After cleaning and washing, individual trace elements were quantified using inductively coupled plasma-mass spectrometry. Sample preparations, quality controls, and measurement of trace elements have been previously described in detail (Amaral *et al.* 2012c).

FGFR3 mutation and expression analysis: Methods of *FGFR3* mutation analysis have been previously reported (Hernandez *et al.* 2005, Hernandez *et al.* 2006). In brief, exon 7 was amplified in all NMIBC cases and exon 10 was amplified in all but nine cases with exon 7 mutations, using PCR. Mutational analysis in MIBC cases was performed using a SNAPShot assay for the 13 most common *FGFR3* mutations (van Oers *et al.* 2006, Hafner *et al.* 2006, Hafner *et al.* 2010).

Details on the FGFR3 expression analysis have been previously reported (Amaral *et al.* 2012b). Briefly, tissue microarrays were made using formalin-fixed paraffin-embedded tissue of the UCB cases recruited to the SBC/EPICURO Study. For immunohistochemistry staining, the monoclonal antibody B9 was used as primary antibody and the Envision+ anti-mouse reagent was applied as secondary antibody (DAKO, Copenhagen, Denmark). The proportion of reactive cells (0-100%) and staining intensity (1+ to 3+) were assessed by a uropathologist. A histoscore was calculated by multiplying the percentage of reactive cells by the intensity. Cut-off definition: high expression was defined by a histoscore >30.

Statistical methods: D4Z4 methylation level from the six CpG sites was averaged. To identify predictors of D4Z4 methylation among the control subjects, robust linear regression models were fitted to estimate unadjusted and multivariable adjusted beta coefficients (βs) and 95% confidence intervals (CIs). The multivariable models were initially adjusted for age, gender, and region, and further adjustment was done by including smoking status into the models. Potential methylation predictors considered were age, gender, region, smoking status (never, occasional, former and current), tobacco type (never, blond tobacco only, black tobacco only, both types and unknown), number of cigarettes smoked per day, duration of cigarette smoking in years, pack-years, nutrient intake, body mass index (<25.0, 25-29.9 and ≥ 30.0), trace elements, and genotypes.

Individuals were subsequently classified into two categories by the median of D4Z4 methylation level (64.4%) among the control subjects. Methylation level below the median was used as the referent category. Continuous covariates were compared between categories (case-control status) by the Mann-Whitney rank test and categorical covariates using the chi-square test. Logistic models, unadjusted and adjusted for age, gender, region, and smoking status, were used to estimate odds ratio (ORs) and 95% CIs for the association between D4Z4 methylation and UCB risk.

We further tested effect modification of the association between D4Z4 methylation and risk of UCB by several non-genetic and genetic factors (e.g, age, gender, smoking, nutrient intakes, toenail trace elements and SNPs in genes involved in the one-carbon metabolism pathway). Interactions between D4Z4 methylation and these factors were tested by including the respective multiplicative interaction term in the multivariable adjusted logistic regression models.

The likelihood ratio test was used to compare the model with and without the interaction term and to calculate the p-value for the interaction. Because 496 SNPs (four modes of inheritance: additive, co-dominant, dominant, recessive) were checked for interaction with D4Z4 methylation, each interaction p-value was corrected for multiple testing using a permutation test. Briefly, this test was done by randomly shuffling the case-control status 10,000 times in a proportion similar to the distribution in the current analysis. The minimum p-value was recorded for each of 10,000 replicates and the corrected chi-square p-values were estimated as the proportion of replication p-values less than the corresponding uncorrected p-value.

Multinomial logistic regression models were applied to assess the association between D4Z4 methylation and the different UCB clinicopathological and molecular subtypes: low-grade NMIBC, high-grade NMIBC, and MIBC; tumors with wild type and mutant *FGFR3*; tumors with low and high FGFR3 expression levels. Adjustment was performed for the same variables included in the logistic regression models. Heterogeneity between the respective risk estimates was assessed using the likelihood ratio test, comparing models with and without the OR constrained to be equal for the corresponding case groups. All tests were two-sided and p-value $\leq$ 0.05 was considered significant. Statistical analyses were performed by STATA/SE version 10.1 (StataCorp, College Station, TX, USA).

Results

The characteristics of the 707 UCB cases and 718 controls included in the present study are shown in Table 5-1. Although not statistically significant, the median (interquartile range) D4Z4 methylation level was slightly higher in cases than in controls [65.6% (13.7) vs. 64.4% (13.6), p-value = 0.2]. The mean (standard deviation) of D4Z4 methylation level among cases and controls were 64.3% (10.1) and 63.6% (9.9), respectively.

Predictors of D4Z4 methylation

The levels of D4Z4 methylation were lower among females (β = -3.3; 95%CI -6.0 to -0.6, p-value = 0.02), smokers of blond tobacco, when compared to never smokers (β = -3.7; 95%CI -6.5 to -0.9, p-value = 0.01), and was strongly inversely associated with toenail selenium concentration (adjusted β for a 1-µg/g increase = -8.1; 95%CI -12.7 to -3.5, p-value = 0.0006) (Table 5-2). On the other hand, arsenic showed a trend towards positive association with D4Z4 methylation levels (adjusted β for a 1-µg/g increase = 13.3; 95%CI -0.9 to 27.4, p-value = 0.07). None of the other potential predictors included in the analyses were significantly associated with D4Z4 methylation level (Supplementary Table 5-1). Results from the multivariable model adjusted for age, gender and region only were not different from the association above (Supplementary Table 5-2).

Table 5-1 Characteristics of controls and cases with D4Z4 methylation data in the Spanish Bladder Cancer/EPICURO Study

Characteristic	Controls (n = 718)	Cases (n = 707)	P-value*
Age in years, median (IQR)	66 (13)	68 (13)	0.0001
Gender, n (%)			
Male	642 (89.4)	617 (87.3)	0.2
Female	76 (10.6)	90 (12.7)	
Region, n (%)			
Barcelona	130 (18.1)	128 (18.1)	0.99
Valles	117 (16.3)	113 (16.0)	
Elche	58 (8.1)	55 (7.8)	
Tenerife	122 (17.0)	120 (17.0)	
Asturias	291 (40.5)	291 (41.1)	
Smoking status, n (%)†			
Never	206 (28.8)	102 (14.5)	<0.0001
Occasional	53 (7.4)	30 (4.3)	
Former	261 (36.5)	266 (38.0)	
Current	195 (27.3)	303 (43.2)	
Tumor type, n (%)‡			
Low-grade NMIBC (TaG1/G2)	–	382 (57.8)	
High-grade NMIBC (TaG3/T1)	–	139 (21.1)	
MIBC (≥T2)	–	139 (21.1)	
FGFR3 expression§			
Low (< 30%)	–	239 (59.3)	
High (≥ 30%)	–	164 (40.7)	
***FGFR3* mutation status¶**			
Wild type	–	313 (59.4)	
Mutant	–	214 (40.6)	
D4Z4 methylation level			
Median (IQR), in %†	64.4 (13.6)	65.6 (13.7)	0.2

IQR, interquartile range; NMIBC, non-muscle-invasive bladder cancer; MIBC, muscle-invasive bladder cancer

*Mann-Whitney test for age and D4Z4 methylation levels; Chi-square test for categorical variables.

† Six cases and three controls have missing information on smoking status.

‡ Forty-two cases could not be assigned to any T-G group because the paraffin blocks could not be retrieved and five cases were carcinoma in situ.

§Three hundred four cases have missing FGFR3 expression data.

¶One hundred eighty cases have missing data on *FGFR3* mutation status.

Table 5-2 Significant predictors of D4Z4 methylation level among the control population in the Spanish Bladder Cancer/EPICURO Study

Characteristic	N	D4Z4 methylation (%)				Unadjusted model				N	Adjusted*			
		Mean	SD	Median	IQR	β	95% CI		P-value		β	95% CI		P-value
Gender														
Male	642	63.9	9.8	64.9	13.8	Reference				639	Reference			
Female	76	61.5	10.4	61.7	11.3	-2.4	-4.8	-0.1	0.04	76	-3.3	-6.0	-0.6	0.02
Tobacco type														
Never	206	63.7	9.9	64.4	12.7	Reference				206	Reference			
Blond tobacco only	82	61.6	11.0	61.4	17.5	-2.3	-4.9	0.3	0.08	82	-3.7	-6.5	-0.9	0.01
Black tobacco only	171	64.3	9.4	65.6	11.5	0.6	-1.4	2.7	0.6	171	-0.4	-2.7	1.8	0.7
Both types	126	63.7	10.1	65.6	13.8	0.2	-2.1	2.4	0.9	126	-0.9	-3.3	1.6	0.5
Unknown	78	63.1	10.3	63.7	14.6	-0.9	-3.5	1.7	0.5	78	-2.2	-5.0	0.6	0.1
Toenail trace element														
Selenium, µg/g	521	—	—	—	—	-4.8	-11.1	1.5	0.1	521	-8.1	-12.7	-3.5	0.0006

SD, standard deviation; IQR, interquartile range.

*Adjusted for age, gender, region, and smoking status (never, occasional, former and current smoker). Beta coefficients of tobacco type are adjusted for age, gender and region.

D4Z4 methylation and risk of UCB

There was a borderline significantly increased risk of UCB among individuals with D4Z4 methylation above or equal to the median compared to those with lower than the median (adjusted OR = 1.23; 95%CI 0.99 - 1.53, p-value = 0.06; Table 5-3). We also assessed whether the methylation level at each of the six CpG positions were individually associated with UCB risk. Similar patterns of risk estimates to the average D4Z4 methylation were observed (Supplementary Table 5-3).

Statistically significant interactions impacting on this association were observed between D4Z4 hypermethylation and toenail iron (p-interaction = 0.003), manganese (p-interaction = 0.04), and zinc (p-interaction = 0.01). The increased risk associated with D4Z4 hypermethylation was found to be significantly higher among subjects with lower levels of toenail iron and manganese and higher toenail levels of zinc (Table 5-4). There was no significant interaction between D4Z4 methylation and age, gender, smoking status, type of tobacco smoked, number of cigarettes smoked per day, duration of cigarette smoking, pack-years, nutrients and nine other trace elements (Supplementary Tables 5-4 and 5-5).

A SNP rs11040387G>A located at the flanking 5' UTR of the folate hydrolase (prostate-specific membrane antigen) 1 (*FOLH1*) significantly modified the effect of increased D4Z4 methylation on UCB risk (p-interaction = 0.02, after correction for multiple testing). There was a 3.5-fold significantly increased risk of UCB among subjects who carried at least one copy of the minor allele (OR = 3.51; 95%CI 1.95 - 6.44, p-value = 5.1×10^{-5}) (Table 5-5). The rest of the SNPs that did not show significant interaction with D4Z4 methylation are shown in Supplementary Table 5-6, with the corresponding uncorrected and corrected p-values for multiple testing.

Table 5-3 Association between D4Z4 methylation level and urothelial carcinoma of the bladder risk stratified by clinicopathological and molecular subphenotypes in the Spanish Bladder Cancer/EPICURO Study

D4Z4 Methylation	Cases	Controls	OR	95% CI		P-value
Overall						
Model 1*						
M1, < 64.4%	328	359	1 Reference			
M2, ≥ 64.4%	379	359	1.16	0.94	1.42	0.2
Model 2†						
M1, < 64.4%	324	358	1 Reference			
M2, ≥ 64.4%	377	357	1.23	0.99	1.53	0.06
Tumor type‡						
Low-grade NMIBC						
M1, < 64.4%	165	358	1 Reference			
M2, ≥ 64.4%	214	357	1.37	1.06	1.78	0.02
High-grade NMIBC						
M1, < 64.4%	65	358	1 Reference			
M2, ≥ 64.4%	71	357	1.17	0.80	1.71	0.4
MIBC						
M1, < 64.4%	73	358	1 Reference			
M2, ≥ 64.4%	66	357	0.97	0.66	1.42	0.9
High FGFR3 expression§						
Low-grade NMIBC						
M1, < 64.4%	46	358	1 Reference			
M2, ≥ 64.4%	71	357	1.63	1.08	2.47	0.02
High-grade NMIBC						
M1, < 64.4%	15	333	1 Reference			
M2, ≥ 64.4%	6	329	0.46	0.17	1.24	0.1
MIBC						
M1, < 64.4%	13	358	1 Reference			
M2, ≥ 64.4%	13	357	1.19	0.52	2.69	0.7

M1, D4Z4 methylation level less than the median; M2, D4Z4 methylation level greater than or equal to the median; NMIBC, non-muscle-invasive bladder cancer; MIBC, muscle-invasive bladder cancer.

*Unadjusted logistic regression model.

†Adjusted for age, gender, region, and smoking status.

‡P value for heterogeneity: low- vs high-grade NMIBC = 0.4; low-grade NMIBC vs MIBC = 0.05; high-grade NMIBC vs MIBC=0.4.

§P value for heterogeneity: low- vs high-grade NMIBC = 0.01; low-grade NMIBC vs MIBC = 0.4; high-grade NMIBC vs MIBC=0.4.

Table 5-4 Association between D4Z4 methylation level and urothelial carcinoma of the bladder risk, by toenail concentrations of iron, manganese, and zinc in the Spanish Bladder Cancer/EPICURO Study

D4Z4 Methylation	Cases	Controls	OR*	95% CI		P-value	P-interaction
Iron (Fe)							
Fe < 14.534 µg/g							
M1, < 64.4%	139	149	1 Reference				
M2, ≥ 64.4%	160	111	1.54	1.08	2.18	0.02	
Fe ≥ 14.534 µg/g							
M1, < 64.4%	121	113	1 Reference				
M2, ≥ 64.4%	137	147	0.94	0.65	1.36	0.7	0.003
Manganese (Mn)							
Mn < 0.333 µg/g							
M1, < 64.4%	139	138	1 Reference				
M2, ≥ 64.4%	176	122	1.47	1.04	2.07	0.03	
Mn ≥ 0.333 µg/g							
M1, < 64.4%	123	124	1 Reference				
M2, ≥ 64.4%	122	137	0.95	0.65	1.37	0.8	0.04
Zinc (Zn)							
Zn < 103.016 µg/g							
M1, < 64.4%	146	125	1 Reference				
M2, ≥ 64.4%	160	136	1.08	0.76	1.53	0.7	
Zn ≥ 103.016 µg/g							
M1, < 64.4%	116	137	1 Reference				
M2, ≥ 64.4%	138	123	1.37	0.95	1.97	0.09	0.01

M1, D4Z4 methylation level less than the median; M2, D4Z4 methylation level greater than or equal to the median.

* Adjusted for age, gender, region, and smoking status (never, occasional, former and current smoker).

D4Z4 methylation and risk of urothelial carcinoma of the bladder subphenotypes

Analyses by tumor subtypes showed differences in the risk associated with higher D4Z4 methylation level. D4Z4 hypermethylation was significantly associated with risk of low-grade NMIBC (OR = 1.37; 95%CI 1.06 - 1.78, p-value = 0.02), and this risk estimate was significantly different from that of MIBC (OR = 0.97; 95%CI 0.66 - 1.42, p-value = 0.9) ($p_{heterogeneity} = 0.05$). Moreover we performed stratified analyses by FGFR3 expression level and mutation status in UCB tissues. In tumors overexpressing FGFR3, the increased risk of developing low-grade NMIBC was markedly elevated by more than 25% (OR = 1.63; 95%CI 1.08 - 2.47, p-value = 0.02), while the odds ratio associated with high-grade NMIBC was 0.46 (95%CI 0.17 - 1.24, p-value = 0.1; $p_{heterogeneity} = 0.01$) (Table 5-3). The association between risk of UCB subtypes and D4Z4 methylation based on *FGFR3* mutation status (wild type and mutant) was not statistically significant (Supplementary Table 5-7).

Table 5-5 Association between D4Z4 methylation level and urothelial carcinoma of the bladder risk, by genotype of a single nucleotide polymorphism in the folate hydrolase (prostate-specific membrane antigen) 1 (*FOLH1*) gene in the Spanish Bladder Cancer/EPICURO Study

D4Z4 Methylation	Cases	Controls	OR*	95% CI		P-value	P-interaction	Corrected p-interaction†
FOLH1 ─ rs11040387[GG]‡								
M1, < 64.4%	273	289	1 Reference					
M2, ≥ 64.4%	287	308	1.02	0.80	1.31	0.9		
FOLH1 ─ rs11040387[GA/AA]								
M1, < 64.4%	41	61	1 Reference					
M2, ≥ 64.4%	79	41	3.51	1.91	6.44	5.1×10^{-5}	7.8×10^{-5}	0.02

M1, D4Z4 methylation level less than the median; M2, D4Z4 methylation level greater than or equal to the median.
* Adjusted for age, gender, region, and smoking status.
† Interaction p-value corrected for multiple testing using a permutation test.
‡ Located at the flanking 5' UTR of *FOLH1* in chromosome 11p11.12 (human genome build 36.3, location 49 450 312). Minor allele frequency = 7%.

Discussion and conclusions

In the present study, we found that gender, type of tobacco and toenail selenium, and probably arsenic concentrations are independent predictors of D4Z4 macrosatellite repeat arrays. We also observed a slightly increased risk of UCB associated with higher levels of D4Z4 methylation and that iron, manganese and zinc concentrations and *FOLH1*-rs11040387 modified such association. In addition, we found that the risk of UCB associated with higher levels of D4Z4 methylation was higher and significantly associated among low-grade NMIBC and tumors overexpressing FGFR3.

Few evidences exist on predictors of global methylation. The lower D4Z4 methylation in females found in the present study are consistent with previous observations of lower level of DNA methylation globally and at other repetitive elements such as LINE-1 and *Alu* (Fuke *et al.* 2004, Wilhelm *et al.* 2010, Hsiung *et al.* 2007, Zhu *et al.* 2012, El-Maarri *et al.* 2007, El-Maarri *et al.* 2011, Zhang *et al.* 2011, Cash *et al.* 2011). The association between blond tobacco smoking and lower D4Z4 methylation is in agreement with an earlier study that showed blond tobacco smokers had lower LINE-1 methylation (see Chapter III). An experimental study investigating the effect of cigarette smoke on the epigenome of bronchial epithelial cells reported that chronic exposure to cigarette smoke condensate resulted in demethylation, not only at D4Z4 repeats, but also at NBL2 and LINE-1 DNA repetitive sequences (Liu *et al.* 2010). These findings suggest that the association of altered DNA methylation with cigarette smoking might be dependent on the type of tobacco smoke. The inverse association between toenail selenium concentration and D4Z4 methylation is in agreement with a study by Pilsner *et al.* who reported an inverse association between plasma selenium and global DNA methylation (Pilsner *et al.* 2011). Selenium is an essential trace element found in the active sites of selenoproteins and its deficiency has been associated with various human diseases, including UCB (Rayman 2012, Amaral *et al.* 2010).

To the best of our knowledge, this is the first study to report an association between D4Z4 methylation in granulocyte DNA and UCB risk. This finding builds upon two previous reports showing that D4Z4 hypermethylation is observed in UCB tissue and cell lines (Choi *et al.* 2009, Cheng *et al.* 2004). D4Z4 tandem repeats located in the subtelomeric region of chromosome 4

have 11-100 units of D4Z4 each 3.3 kilobase long. Each repeat unit contains a copy of *DUX4* gene, which encodes a transcription factor and a well studied candidate gene for FSHD (Geng *et al.* 2012). It has been shown that *DUX4* regulates a host of genes that are involved in various cellular processes including oncogenesis, immune function and apoptosis (Geng *et al.* 2012). Additionally, it has been demonstrated that *DUX4* activates the transcriptional factor paired-like homeodomain 1 (*PITX1*) gene located in chromosome 5q31.1 (Dixit *et al.* 2007). *PITX1* is a tumor-suppressor gene that has been shown to negatively regulate telomerase reverse transcriptase (*TERT*) gene (Qi *et al.* 2011), and *RAS* pathway to inhibit tumorigenesis (Kolfschoten *et al.* 2005). Of relevance, a recent genome-wide association study identified a common variant at *TERT* gene as a susceptibility locus to UCB (Rothman *et al.* 2010). Similarly, *PITX1* has been also shown to directly activate *TP53*, itself a tumor-suppressor gene (Liu & Lobie 2007). It has been established that *RAS* and *TP53* pathways are frequently altered in NMIBC and MIBC, respectively (Cheng *et al.* 2011, Knowles 2008, Falke & Witjes 2011), and reduced *PITX1* expression has been found in bladder and other cancer types (Kolfschoten *et al.* 2005). *DUX4* expression is regulated by DNA methylation and chromatin structures (van der Maarel *et al.* 2012), and hypermethylation of D4Z4 has been shown to be inversely correlated with *DUX4* expression in human papillomavirus-positive cervical cancer tissues (Katargin *et al.* 2009). This indicates that, by suppression of *DUX4* expression, D4Z4 hypermethylation might result in UCB through dysregulated expression of *DUX4* target genes such as *PITX1*.The observation in the current study of DNA hypermethylation in D4Z4 repeats associated with increased risk suggests that D4Z4 methylation in peripheral blood cells could be used as a biomarker of UCB susceptibility.

We also found that the effect of D4Z4 hypermethylation was modified by both environmental and genetic factors. Specifically iron, manganese, and zinc modified the risk conferred by increased D4Z4 methylation. The mechanism through which these trace elements could modify the risk is unknown. Iron, manganese and zinc are essential minerals, important for cellular homeostasis and protection from oxidative stress (Bleackley & Macgillivray 2011, Aguirre & Culotta 2012). Recently, it has been suggested that both acute and chronic oxidative stress can induce carcinogenesis through altered DNA methylation and other epigenetic modification (Johnstone & Baylin 2010). Iron, together with α-ketoglutarate, is an important cofactor for the ten-eleven translocation (*TET1*, *TET2*, *TET3*) family of DNA hydroxylases that

convert 5-methylcytosine to 5-hydroxymethylcytosine, a mechanism implicated in active and passive DNA demethylation (Tahiliani *et al.* 2009, Ito *et al.* 2011). We have recently shown that iron is associated with DNA methylation level in LINE-1 repetitive DNA sequences (see Chapter III). Like iron and other essential nutrients, zinc also modulates the epigenetic landscape. Betaine-homocysteine S-methyltransferase requires zinc as a cofactor to catalyze the transfer of methyl group from betaine to homocysteine to produce methionine, a precursor of S-adenosyl methionine (Ulrey *et al.* 2005). It has been demonstrated that zinc deficiency caused DNA and histone hypomethylation in rat liver (Wallwork & Duerre 1985).

Furthermore, an interaction between D4Z4 methylation and rs11040387 located in the flanking 5' UTR of *FOLH1* was also observed in the present study. *FOLH1*, also known as glutamate carboxypeptidase II (Gene ID: 2346), is a type II transmembrane glycoprotein expressed in the membrane brush boarder of the proximal small intestine where it regulates folate absorption by catalyzing the hydrolysis of folate poly-γ-glutamates into monoglutamate derivatives (Heston 1997). A missense mutation in *FOLH1* has been associated with abnormal plasma folate and total homocysteine concentrations, and altered enzyme activity (Halsted *et al.* 2007), mechanisms through which common variations at this gene could result in impaired one-carbon metabolism. *FOLH1* is also found to be overexpressed in a multitude of solid tumors and their neovasculatures including UCB (Samplaski *et al.* 2011) where it is suggested to regulate angiogenesis by increasing the local concentration of folate, which in turn increases the production of the proangiogenic molecule nitric oxide by stimulating regeneration of the endothelial nitric oxide synthase cofactor, tetrahydrobiopterin (Gordon *et al.* 2008). In prostate cancer, overexpression is associated with advanced disease stage and poor prognosis (Samplaski *et al.* 2011). The SNP discussed above has not been previously reported in relation to UCB.

The limitations of this study include the fact that blood samples were collected at time of diagnosis, although before the interview and initiation of treatment. The fact that most of UCB does not affect general performance supports the use of D4Z4 methylation as a long-term marker. Not all study subjects from the SBC/EPICURO Study had their D4Z4 methylation levels measured, and controls with this data were slightly younger and over-represented by males. However, comparing cases with and without D4Z4 methylation showed no significant differences between them (Supplementary Table 5-8). Importantly, the study also has important strengths.

Two of these are its large sample size and the multi-level integration of data including epidemiologic, clinical, environmental and genetic variables. Another strength of the study was the choice of pyrosequencing as the method to quantify D4Z4 methylation levels, which seems to provide highly reproducible and accurate results (Tost & Gut 2007). Samples were run in duplicates and the within sample coefficient of variation was 0.88%.

To our knowledge, this is the first study showing that gender, tobacco type and selenium may independently predict the levels of D4Z4 methylation, and that D4Z4 hypermethylation increases the risk of low-grade NMIBC and FGFR3 overexpressing tumors. In addition, it is shown that the association between D4Z4 hypermethylation and risk of UCB can be modified by non-genetic (iron, manganese, and zinc) and genetic (*FOLH1*-rs11040387) factors. Therefore, measuring D4Z4 methylation in peripheral blood could be a potential biomarker of UCB susceptibility. The fact that D4Z4 hypermethylation is associated with specific UCB subtyphenotypes characterized by clinicopathological and molecular markers might imply the presence of a D4Z4 methylator phenotype of UCB. Further studies are required in larger populations to extend and replicate our findings. Moreover, experimental studies should be conducted to elucidate the consequences of D4Z4 hypermethylation in the bladder carcinogenesis.

CHAPTER VI. LACK OF ASSOCIATION BETWEEN GENOMIC DNA METHYLATION IN LEUKOCYTE DNA AND PROGNOSIS OF UROTHELIAL CARCINOMA OF THE BLADDER

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Abstract

Altered DNA methylation is an early event in the development of urothelial carcinoma of the bladder (UCB). We hypothesized that it might also impact on disease progression and assessed the association between genomic DNA methylation in granulocytes and UCB outcomes in the Spanish Bladder Cancer/EPICURO study. Methylation levels in DNA repeats - LINE-1 (N = 895) and D4Z4 (N = 665) - were measured by using pyrosequencing in newly diagnosed UCB patients that were followed-up for over 10 years. Primary diagnosis were homogeneously confirmed and classified by a panel of expert pathologists. Clinical and treatment data were collected from hospital charts. Association between methylation and UCB outcomes (recurrence, progression, relapse, mortality) was assessed by using unadjusted and adjusted Cox proportional hazards regression models. Neither LINE-1 nor D4Z4 methylation levels were significantly associated with recurrence, progression, or relapse in non-muscle-invasive bladder cancer patients, and progression, bladder cancer-specific or overall mortality in muscle-invasive bladder cancer patients. This is the first study to assess the association between LINE-1 and D4Z4 methylation and UCB outcomes. The results of do not support genomic DNA methylation in granulocytes as an independent prognostic factor in UCB patients.

Introduction

Patients with urothelial carcinoma of the bladder (UCB) present as non-muscle-invasive (NMIBC, 80%) or muscle-invasive bladder cancer (MIBC, 20%) at the time of diagnosis. NMIBC is characterized by increased rate of recurrence, few experiencing progression to muscle-invasive disease. Clinicopathological parameters are commonly used for prognostication of the disease. However, they lack accuracy and novel markers are needed to improve prognostic accuracy (Netto 2012). Aberrant DNA methylation occurs early in the development of several cancer types including UCB and is considered a hallmark of cancer (Kim & Kim 2012). Hypomethylation of retrotransposons such as long interspersed nuclear elements 1 (LINE-1) and hypermethylation of the subtelomeric tandem repeat element D4Z4 have been observed in UCB (Neuhausen *et al.* 2006, Choi *et al.* 2009). Moreover, LINE-1 hypomethylation and promoter hypermethylation of specific genes in the tumor tissue have been shown to associate with significantly shorter UCB recurrence-free and disease-specific survival time (Kim & Kim 2012, Neuhausen *et al.* 2006).

Altered DNA methylation in peripheral blood cells has been associated with elevated risk of developing UCB (Moore *et al.* 2008, Wilhelm *et al.* 2010, Cash *et al.* 2012). However, its effect on disease progression and mortality has not been studied. Here, we assessed the association between methylation levels at LINE-1 and D4Z4 sequences measured in granulocyte DNA and UCB outcomes, and whether this association is modified by selected factors.

Methods

Details of the study population, design, data collection, genotyping have been described elsewhere (Garcia-Closas *et al.* 2005, Rothman *et al.* 2010). After written informed consent and institutional review board approval, blood was drawn for DNA extraction at the time of diagnosis and before treatment. Patients were incident cases of UCB recruited from non-referral hospitals located in five regions of Spain. Diagnosis, tumor grade and stage were confirmed by a panel of expert pathologists. During follow-up, data on vital status, recurrence, progression and relapse were collected by trained nurses through telephone interviews and individual patient's hospital charts. By pyrosequencing, the methylation levels of four CpG sites at LINE-1 and six CpG sites at D4Z4 elements were measured (see chapters III and V for details of methylation

quantifications). The average methylation level of the CpG sites for each repeat sequence was used in the final analysis.

We used Cox proportional hazards regression models to estimate hazard ratios (HRs) and 95% confidence intervals (95%CI) for each outcome: recurrence, progression, and relapse (event-free survival) in NMIBC, and progression, disease-specific, and overall mortality in MIBC. LINE-1 methylation level was modeled as: (i) a continuous variable; (ii) categorized by the median; and (iii) in tertiles. D4Z4 methylation was modeled as: (i) a continuous variable and (ii) categorized by the median. Methylation level less than the median value (LINE-1 and D4Z4) and the middle tertile (LINE-1) were used as the reference group. Multiplicative interaction between DNA methylation and selected genetic and non-genetic variables was also assessed. We checked the Cox proportional hazards assumptions by plotting log-log survival curves for LINE-1 and D4Z4 methylation levels, by performing the time-dependent covariate test, and the scaled Schoenfeld residuals method. The proportional hazard assumptions were not violated (*P* value >0.05). *P* values less than or equal to 0.05 were considered to indicate statistical significance. All tests were two-sided. STATA/SE software, version 10.1 (StataCorp, College Station, Texas) was used for all statistical analyses. This study had an estimated power of 80% to detect HRs of 1.25 and 1.40 associated with a methylation level above the median for all outcomes considered in NMIBC and MIBC patients, respectively. Power estimation was performed using Epi Info software, version 7.1.1.14 (CDC, Atlanta, Georgia).

Results

Patient characteristic stratified by LINE-1 and D4Z4 methylation levels are shown in Supplementary Tables S6-1 and S6-2. Median follow-up time for NMIBC patients with LINE-1 and D4Z4 data was 76.1 and 76.2 months, and for MIBC patients was 23.3 and 25.5 months, respectively. In NMIBC patients, characteristics were similarly distributed across the different strata of LINE-1 methylation and D4Z4 methylation, except age which was directly associated with LINE-1 methylation. Characteristics of MIBC patients were also similarly distributed, with the exception of age and sex that were associated with D4Z4 methylation.

LINE-1 methylation was not associated with outcomes in both NMIBC and MIBC patients in unadjusted and adjusted Cox regression models (Table 6-1). Likewise, D4Z4 methylation was not associated with outcomes in both NMIBC and MIBC patients (Table 6-2).

Stratified analyses by *FGFR3* mutation levels and expression status were also nonsignificant (data not shown). Furthermore, we found no effect modification by each of the following: smoking status, tobacco type, nutrient intakes, combined tumor stage and grade, type of treatment, 461 single nucleotide polymorphisms in 24 one-carbon metabolism pathway genes, *NAT2* acetylation phenotype (rs1495741), *GSTM1* and *GSTT1* copy number variations, and UCB susceptibility loci (data not shown) (Rothman *et al.* 2010).

Table 6-1 Association between LINE-1 methylation in leukocyte DNA and outcomes of urothelial carcinoma of the bladder in the Spanish Bladder Cancer/EPICURO study

LINE-1 methylation*	No. of Events	Total No. of Patients	HR (95% CI)†	P-Value	No. of Events	Total No. of Patients	Adjusted HR (95% CI)‡	P-Value
NMIBC								
Recurrence								
Continuous	231	706	1.00 (0.97-1.02)	0.7	216	655	0.99 (0.97-1.02)	0.5
M1	121	353	1 Reference		116	329	1 Reference	
M2	110	353	0.88 (0.68-1.14)	0.3	100	326	0.82 (0.62-1.08)	0.2
T1	83	236	1.20 (0.88-1.65)	0.2	81	221	1.40 (1.00-1.95)	0.05
T2	73	235	1 Reference		66	216	1 Reference	
T3	75	235	1.05 (0.76-1.45)	0.8	69	218	1.06 (0.75-1.50)	0.7
Progression								
Continuous	66	706	1.01 (0.98-1.05)	0.5	58	667	1.02 (0.98-1.07)	0.3
M1	29	353	1 Reference		26	334	1 Reference	
M2	37	353	1.29 (0.79-2.10)	0.3	32	333	1.29 (0.75-2.20)	0.4
T1	20	236	1.11 (0.59-2.10)	0.7	18	224	1.52 (0.74-3.13)	0.3
T2	18	235	1 Reference		14	219	1 Reference	
T3	28	235	1.59 (0.88-2.88)	0.1	26	224	2.28 (1.15-4.51)	0.02
Relapse								
Continuous	277	706	0.99 (0.98-1.02)	0.6	256	667	0.99 (0.97-1.02)	0.6
M1	143	353	1 Reference		135	334	1 Reference	
M2	134	353	0.91 (0.72-1.15)	0.4	121	333	0.88 (0.68-1.12)	0.3
T1	99	236	1.22 (0.92-1.63)	0.2	95	224	1.43 (1.05-1.94)	0.02
T2	86	235	1 Reference		76	219	1 Reference	
T3	92	235	1.10 (0.82-1.47)	0.5	85	224	1.18 (0.86-1.62)	0.3
MIBC								
Progression								
Continuous	108	189	1.01 (0.98-1.03)	0.7	108	189	1.01 (0.98-1.04)	0.4
M1	52	95	1 Reference		52	95	1 Reference	
M2	56	94	1.05 (0.72-1.53)	0.8	56	94	0.89 (0.60-1.33)	0.6

Table 6-1 (cont.) Association between LINE-1 methylation in leukocyte DNA and outcomes of urothelial carcinoma of the bladder in the Spanish Bladder Cancer/EPICURO study

LINE-1 Methylation*	No. of Events	Total No. of Patients	HR (95% CI)†	P-Value	No. of Events	Total No. of Patients	Adjusted HR (95% CI)‡	P-Value
T1	33	63	0.86 (0.54-1.37)	0.5	33	63	0.90 (0.53-1.51)	0.7
T2	39	63	1 Reference		39	63	1 Reference	
T3	36	63	0.93 (0.59-1.47)	0.8	36	63	0.76 (0.46-1.26)	0.3
BCSM								
Continuous	91	189	1.00 (0.97-1.03)	0.9	91	189	1.01 (0.98-1.04)	0.6
M1	42	95	1 Reference		42	95	1 Reference	
M2	49	94	1.17 (0.78-1.77)	0.4	49	94	1.06 (0.68-1.64)	0.8
T1	31	63	1.18 (0.71-1.97)	0.5	31	63	1.10 (0.63-1.94)	0.7
T2	28	63	1 Reference		28	63	1 Reference	
T3	32	63	1.21 (0.73-2.01)	0.5	32	63	1.00 (0.57-1.75)	1.0
OM								
Continuous	129	189	0.99 (0.97-1.02)	0.6	129	189	1.00 (0.97-1.02)	0.9
M1	64	95	1 Reference		64	95	1 Reference	
M2	65	94	1.03 (0.73-1.45)	0.2	65	94	0.85 (0.59-1.23)	0.4
T1	43	63	1.07 (0.70-1.63)	0.8	43	63	1.03 (0.65-1.63)	0.9
T2	42	63	1 Reference		42	63	1 Reference	
T3	44	63	1.09 (0.71-1.66)	0.7	44	63	0.86 (0.54-1.37)	0.5

HR, hazard ratio; Ref, referent; NMIBC, non-muscle invasive bladder cancer; MIBC, muscle invasive bladder cancer; M, median; T, tertile; BCSM, bladder cancer-specific mortality; OM, overall mortality.

*Cutoff values used to categorize LINE-1 methylation in medians (57.6%), tertiles (56.6 and 58.9%) in NMIBC cases, and median (57.7%) and tertile (56.7 and 59.5%) in MIBC cases.

†Crude model.

‡Models adjusted for commonly used prognostic factors for urothelial carcinoma of the bladder. NMIBC recurrence adjusted for sex, residential area, tumor stage and grade, tumor size, multiplicity, and treatment; NMIBC progression adjusted for age, residential area, tumor stage and grade, multiplicity, number of recurrences, and treatment; NMIBC relapse adjusted for age, residential area, tumor stage and grade, tumor size, multiplicity, and treatment; MIBC progression adjusted for residential area, tumor stage, nodal metastases, and treatment; MIBC disease specific and overall survival adjusted for residential area, tumor stage, nodal metastases, distant metastases, and treatment.

Table 6-2 Association between D4Z4 methylation in leukocyte DNA and outcomes of urothelial carcinoma of the bladder in the Spanish Bladder Cancer/EPICURO study

D4Z4 Methylation*	No. of Events	Total No. of Patients	HR (95% CI)†	P-Value	No. of Events	Total No. of Patients	Adjusted HR (95% CI)‡	P-Value
NMIBC								
Recurrence								
Continuous	174	526	1.01 (0.998-1.03)	0.09	163	487	1.01 (0.99-1.03)	0.2
M1	78	263	1 Reference		75	240	1 Reference	
M2	96	263	1.29 (0.96-1.74)	0.1	88	247	1.20 (0.87-1.64)	0.3
Progression								
Continuous	50	526	1.00 (0.98-1.03)	0.8	43	494	1.01 (0.98-1.04)	0.6
M1	25	263	1 Reference		20	245	1 Reference	
M2	25	263	0.99 (0.57-1.73)	1.0	23	249	1.28 (0.68-2.41)	0.5
Relapse								
Continuous	207	526	1.01 (0.997-1.02)	0.1	191	494	1.01 (0.996-1.03)	0.2
M1	96	263	1 Reference		88	245	1 Reference	
M2	111	263	1.21 (0.92-1.59)	0.2	103	249	1.24 (0.92-1.66)	0.2
MIBC								
Progression								
Continuous	75	139	1.00 (0.98-1.02)	0.9	75	139	0.99 (0.97-1.02)	0.5
M1	37	70	1 Reference		37	70	1 Reference	
M2	38	69	0.99 (0.63-1.56)	1.0	38	69	0.95 (0.57-1.58)	0.8

Table 6-2 (cont.) Association between D4Z4 methylation in leukocyte DNA and outcomes of urothelial carcinoma of the bladder in the Spanish Bladder Cancer/EPICURO study

D4Z4 Methylation*	No. of Events	Total No. of Patients	HR (95% CI)†	P-Value	No. of Events	Total No. of Patients	Adjusted HR (95% CI)‡	P-Value
MIBC								
BCSM								
Continuous	65	139	1.00 (0.98-1.03)	0.8	65	139	0.99 (0.96-1.02)	0.4
M1	30	70	1 Reference		30	70	1 Reference	
M2	35	69	1.13 (0.69-1.83)	0.3	35	69	1.00 (0.59-1.70)	1.0
OM								
Continuous	94	139	1.00 (0.97-1.02)	0.7	94	139	0.98 (0.96-1.01)	0.2
M1	45	70	1 Reference		45	70	1 Reference	
M2	49	69	1.03 (0.69-1.55)	0.9	49	69	0.96 (0.62-1.48)	0.9

HR, hazard ratio; Ref, referent; NMIBC, non-muscle-invasive bladder cancer; MIBC, muscle-invasive bladder cancer; M, median; BCSM, bladder cancer-specific mortality; OM, overall mortality.

*Cutoff values used to categorize D4Z4 methylation in medians 65.8% in NMIBC cases, and 63.9% in MIBC cases.

†Crude model.

‡Models adjusted for commonly used prognostic factors for urothelial carcinoma of the bladder. NMIBC recurrence adjusted for sex, residential area, tumor stage and grade, tumor size, multiplicity, and treatment; NMIBC progression adjusted for age, residential area, tumor stage and grade, multiplicity, number of recurrences, and treatment; NMIBC relapse adjusted for age, residential area, tumor stage and grade, tumor size, multiplicity, and treatment; MIBC progression adjusted for residential area, tumor stage, nodal metastases, and treatment; MIBC disease specific and overall survival adjusted for residential area, tumor stage, nodal metastases, distant metastases, and treatment.

Discussion and conclusions

In this study, we aimed to assess the association between genomic DNA methylation and UCB outcomes and the potential effect modification by factors that contribute to UCB development and prognosis. Neither LINE-1 nor D4Z4 methylation was associated with any outcome in NMIBC and MIBC. Although previous studies have reported significant associations between tumor tissue LINE-1 and gene promoter methylation and UCB survival (Neuhausen *et al.* 2006, Kim & Kim 2012), the present findings do not support the use of LINE-1 and D4Z4 methylation levels in neutrophils as independent prognostic markers for UCB. While this is a large study, it is the first to assess the association between genomic LINE-1 and D4Z4 methylation and UCB survival. Therefore, independent studies are required to confirm the null results on DNA methylation in peripheral blood cells as a potential prognostic marker for UCB.

CHAPTER VII. GENERAL DISCUSSION

DNA methylation and other epigenetic modifications are important for normal cellular differentiation and development. These modifications are influenced by both the internal and external environment; when these modifications go awry, they result in phenotypic and physiological changes potentially altering the risk of some diseases (Tammen *et al.* 2012). In brief, the objectives of this thesis were to identify potential genetic and non-genetic predictors of genomic DNA methylation at LINE-1 and D4Z4 repeat sequences in granulocytes; to assess the association between genomic DNA methylation and risk of UCB; and to determine the prognostic role of genomic DNA methylation on UCB outcomes. This thesis also aimed at evaluating effect modifications on both disease risk and survival by known and potential risk factors of UCB.

Gender, tobacco type, trace elements (arsenic, iron, and nickel), and some single nucleotide polymorphisms (SNPs) in one-carbon metabolism pathway genes (*DNMT3A*, *AS3MT*, *MTHFS*, *SLC19A1*, and *TCN2*) were associated with levels of LINE-1. D4Z4 methylation levels were associated with gender, tobacco type and selenium. Females presented with lower levels of both LINE-1 and D4Z4 methylation levels. Arsenic, iron, and nickel were associated with LINE-1 methylation, the latter two positively. Selenium was inversely associated with D4Z4 methylation levels. Among common genetic variants evaluated, seven SNPs (*DNMT3A*-rs7581217, *AS3MT*-rs7085104, *MTHFS*-rs1380642, *SLC19A1*-rs914238, *TCN2*-rs9621049, *TCN2*-rs9606756, and *TCN2*-rs4820887) showed association with LINE-1 methylation levels. Regarding risk of UCB, LINE-1 showed a U-shaped association, which was modified by five genetic variants (rs2124344, rs7215833, rs4646340, rs4646350, and rs4646341) at phosphatidylethanolamine *N*-methyltransferase (*PEMT*). D4Z4 hypermethylation was associated with low-grade NMIBC overexpressing FGFR3. The effect of D4Z4 methylation on the overall risk of UCB was modified by iron, manganese, zinc, and rs11040387 in folate hydrolase (prostate-specific membrane antigen) 1 (*FOLH1*). Neither LINE-1 nor D4Z4 methylation was associated with any of the outcomes of UCB.

7.1. Predictors of LINE-1 and D4Z4 methylation

Compared to males, females had a 0.7% and 3.3% decreased level of LINE-1 and D4Z4 methylation, respectively. Increasing studies have shown that females have significantly lower levels of LINE-1 methylation than males (Cash *et al.* 2011, Cash *et al.* 2012, El-Maarri *et al.* 2007, El-Maarri *et al.* 2011, Fuke *et al.* 2004, Hsiung *et al.* 2007, Subramanyam *et al.* 2013, Wilhelm *et al.* 2010, Zhang *et al.* 2011, Zhu *et al.* 2012, Terry *et al.* 2011). The precise explanations for this observation are unknown. DNA methylation is one of the epigenetic mechanisms used to inactivate one of the two X chromosomes to ensure gene dosage compensation. LINE-1 sequences are thought to participate in this mechanism (Bailey *et al.* 2000). Therefore, one would expect females to have higher levels of methylation than males. However, studies both in humans (peripheral blood cells) and mice have shown that transcriptionally active LINE-1 elements can be hypomethylated in the inactive X-chromosome, and that the presence of full-length active LINE-1 element may be correlated with the degree of inactivation (Singer *et al.* 2012, Chow *et al.* 2010). Given that active LINE-1s have full promoters at their 5'untranslated region (UTR) end and the pyrosequencing method detects those LINE-1s with full promoters (see Methods section in chapter III), could these active LINE-1s on the X chromosome correspond to the ones detected by pyrosequencing on the X chromosome, and hence their low methylation level? Further experimental studies may provide direct answer to this question. The study by Singer *et al.* also assessed the effect of chromosomal constitution on LINE-1 methylation by including individuals with numerical X chromosome abnormalities [Turner syndrome (45,X), and Klinefelter syndrome (47,XXY)] in their analysis. There was an inverse association between methylation and genome size, with samples from Klinefelter patients having the lowest LINE-1 methylation level, suggesting limited methylation capability in bigger genomes (Singer *et al.* 2012). Another potential explanation is the differences in male and female nutrient intake and loss, particularly of nutrients such as folate, vitamins B_6 and B_{12}, methionine, and choline. These nutrients are critical substrates of the one-carbon metabolism pathway that generate the methyl group donor - *S*-adenosylmethionine (SAM) - used in DNA methylation reactions (Hsiung *et al.* 2007). An earlier study by Poirier *et al.* comparing blood levels of SAM among males and females showed that the latter had a 40% decreased level of SAM as compared to the former (Poirier *et al.* 2001). An alternative explanation put forward to account for the

difference in LINE-1 methylation between males and females relates to hormonal factors. A study conducted on human cell lines treated with estrogen, progesterone and dihydrotestosterone showed that these hormones did not have any effect on DNA methylation level (El-Maarri *et al.* 2011). There is no published data on D4Z4 methylation to compare the results of D4Z4 methylation between genders. Some of the explanations described above, such as that of the one-carbon metabolism-related nutrients, may contribute to this difference in D4Z4 methylation. Further investigations may shade light on the underlying mechanisms.

In the present work, tobacco type was a significant predictor of both LINE-1 and D4Z4 methylation. Compared to never smokers, smokers of blond tobacco presented a 0.7% and 3.7% decreased LINE-1 and D4Z4 methylation, respectively, whereas smoking black tobacco showed no significant association. These results suggest that the effect of smoking on DNA methylation could be dependent of the type of tobacco (blond vs. black) and that blond tobacco exert its effect through modulating the epigenome. Previous studies assessing the effect of smoking on global DNA methylation reported no evidence of association. However, these studies did not evaluate the effect of the type of tobacco smoked (Terry *et al.* 2011). An experimental study assessing the effect of tobacco smoke condensate on methylation pattern of respiratory epithelial cells found time dependent DNA demethylation in LINE-1, D4Z4, and NABL2 repeat sequences. Evidence from a bladder cancer study showed that smokers of blond tobacco who quit smoking several years before diagnosis were able to approximate their risk to that of never smokers (Samanic *et al.* 2006), suggesting a potentially reversible mechanism for blond tobacco-mediated carcinogenesis. The observation in this study related the influence of blond tobacco on DNA methylation may lend some mechanistic insight into the effects of tobacco type and smoking cessation. Given tobacco smoking is amenable to behavioral and medical interventions and that DNA methylation is a reversible epigenetic modification, these results may have relevant public health implications.

Of the twelve trace elements assessed in this study, arsenic, nickel, iron, and selenium showed significant associations with LINE-1 and D4Z4 methylation. Arsenic was inversely associated with LINE-1 methylation, whereas iron and nickel were positively associated. Selenium was inversely associated with D4Z4 methylation. For 1-μg/g increase in toenail arsenic concentration there was a 3.6% decrease in LINE-1 methylation level. This finding is in

agreement with previous results from human and laboratory studies on arsenic (Wilhelm *et al.* 2010, Reichard & Puga 2010, Ren *et al.* 2011). Although the exact mechanisms through which arsenic bring about DNA methylation changes are not known, some potential explanations have been put forward. Arsenic is a known carcinogen and it is biotransformed by a sequential oxidative-methylation and reduction reactions by arsenic (+3 oxidation state) methyltransferase (AS3MT). This process utilizes multiple equivalents of SAM per molecule of arsenic to produce monomethylarsonic acid and dimethylarsinic acid before excretion through urine (Ren *et al.* 2011, Reichard & Puga 2010). These reactions also result in the accumulation of *S*-adenosylhomocysteine (SAH). This consumption of SAM as well as feedback inhibition of DNA methyltransferases (DNMTs) by SAH has been proposed as a potential mechanism (Reichard & Puga 2010). In addition, arsenic has been shown to directly interact with DNMTs and inhibit their activities (Ren *et al.* 2011). Therefore, by competing for SAM and inhibiting DNMTs, arsenic might lead to decreased LINE-1 methylation levels.

Nickel was positively associated with LINE-1 methylation. The mechanism(s) through which nickel affects DNA methylation is far from clear. Results from studies conducted with cell lines, which are in agreement with the findings from the current study, suggest that nickel treatment induces global DNA methylation and chromatin structural changes (Lee *et al.* 1998, Lee *et al.* 1995). Likewise, iron was also positively associated with LINE-1 methylation. Iron is a critical element in the function of all cells, including transport proteins and enzymes such as oxygenases. Iron is an essential cofactor for 2-oxoglutarate (alpha-ketoglutarate) - dependent ten-eleven translocation (TET1, TET2, TET3) family of methylcytosine hydroxylases, which have been suggested to play a role in DNA demethylation (Bhutani *et al.* 2011, Wu & Zhang 2010). These enzymes have recently been shown to sequentially oxidize 5-methylcytosine into 5-hydroxymethylcytosine, 5-formylcytosine, and 5-carboxylcytosine, which might represent new epigenetic states or intermediates in the DNA demethylation process (Tahiliani *et al.* 2009, Ito *et al.* 2011, Wu & Zhang 2011). Furthermore, hereditary hemochromatosis, a disease characterized by longstanding iron overload, has been shown to be associated with hypermethylation of critical genes in apoptosis and cell cycle (Lehmann *et al.* 2007). These results indicate the role of iron in epigenetic modifications.

D4Z4 methylation was inversely and strongly associated with selenium. For each increase in 1-μg/g of selenium there was an 8% decrease in D4Z4 methylation. This is consistent with a study that showed inverse association between selenium and the total genomic 5-methylcytosine content (Pilsner *et al.* 2011). Selenium is another essential element, critical for normal function of selenoproteins that have anti-oxidant and anti-inflammatory effects. Selenium deficiency has been associated with UCB and many other chronic diseases (Rayman 2012, Amaral *et al.* 2010). The mechanism by which selenium alters DNA methylation patterns is currently unclear and results from animal and *in vitro* studies have been inconsistent. Studies in cancer cell lines showed that treatment with selenium resulted in genomic DNA hypomethylation, inhibition, and down-regulation of DNMTs (Xiang *et al.* 2008, Fiala *et al.* 1998, Cox & Goorha 1986). It is likely that the observed association in this study might be the result of decreased DNMTs activity.

This project also identified seven common germline variants that have not been previously reported before in relation to DNA methylation changes. These genetic variants were located in five genes – *AS3MT*, *DNMT3A*, *MTHFS*, *SLC19A1*, and *TCN2*; three SNPs were located in *TCN2*, and the rest were each located in one of the other four genes. Two of the three SNPs in *TCN2* were missense variants: rs9606756 located at exon 2 results in isoleucine to valine substitution at position 23; and rs9621049 located in exon 7 results in serine to phenylalanine change at position 348. SNPs at *AS3MT*-rs7085104 and *SLC19A1*-rs914238 are located at the 5'UTR, whereas *MTHFS*-rs1380642 is located at 3'UTR. *TCN2*-rs9606756 falls in the NAGNAG tandem acceptor site suggesting that it can have an effect on the alternative splicing of this gene (Hiller *et al.* 2006). Genetic variants located at regulatory sites such as 5'UTR and 3'UTR may alter gene expression, interfere with mRNA stability and translation through effects on polyadenylation and regulatory protein-mRNA and microRNAs-mRNA interactions (Skeeles *et al.* 2013). Altogether, these variants, through alteration of transcription and translation, may affect the one-carbon metabolism pathway, and therefore DNA methylation.

7.2. DNA methylation and UCB risk and survival

The effects of genomic DNA methylation at LINE-1 and D4Z4 elements on risk of UCB were also evaluated in this work. The results showed a U-shaped association between LINE-1 methylation and UCB risk, that is, both low and high LINE-1 methylation levels were associated with a 30% increased risk. This association was significantly modified by variants in the one-carbon metabolism gene *PEMT*. D4Z4 hypermethylation was associated with increased risk of UCB particularly in low-grade NMIBC with FGFR3 overexpression.

Altered global DNA methylation in tumors occurs quite frequently and epidemiologic studies in peripheral blood cells have shown that both hypo- and hypermethylation may be associated with increased risk of cancer (Moore *et al.* 2008, Wilhelm *et al.* 2010, Cash *et al.* 2012, Liao *et al.* 2011). Three previous studies conducted on global DNA methylation and UCB risk showed an inverse association (Moore *et al.* 2008, Wilhelm *et al.* 2010, Cash *et al.* 2012). The study by Cash *et al*. found a significant inverse association between UCB and LINE-1 methylation only among never smokers, whereas the study by Wilhelm *et al*. showed the effect was stronger among females than males; both studies used the same assay to measure LINE-1 methylation as in the present study. The observation that individuals in the lowest tertile have increased risk is consistent with these findings. However, the present study also showed levels of methylation in the highest tertile were associated with increased risk, which is in agreement with a study by Liao *et al*. showing a direct association between LINE-1 methylation and increased risk, albeit in renal cancer (Liao *et al.* 2011). U-shaped associations in cancer are not uncommon, as these have been reported elsewhere, for example, between folate and pancreatic cancer, telomere length and pancreatic cancer, serum vitamin D and prostate cancer, and serum sex hormones and prostate cancer (Chuang *et al.* 2011, Skinner *et al.* 2012, Tuohimaa *et al.* 2004, Salonia *et al.* 2012). How abnormal LINE-1 methylation level results in a nonlinear risk pattern on UCB is not known? It is known that the effect of DNA methylation is dependent on the genomic context it is located at (Jones 2012). This implies that the methylation pattern in LINE-1 elements could have a differential effect depending on their location in the genome. Hypomethylated LINE-1 promoter can induce expression of *MET* oncogene in bladder tumors and other cancer types (Wolff *et al.* 2010, Weber *et al.* 2010), whereas methylated repetitive

elements such as LINE-1 located in gene bodies have been shown to facilitate gene expression and transcriptional elongation of the host gene (Jones 2012). Another explanation is that the U-shaped association could be due to confounding by important risk factors of UCB such as smoking status, tobacco type and gender. However, adjustment with potential and other known risk factors of UCB did not change this association. Furthermore, stratified analysis showed that the observed association did not differ across smoking status, tobacco type or gender strata.

Finally, differences between the present and the previous two case-control studies on LINE-1 methylation and UCB risk could be explained by such factors as the cell type for DNA extraction, the heterogeneity between assays, and difference in sample size. Regarding DNA source, each study used different cell types; one study used lymphocytes only (Cash *et al.* 2012), the other used buffy coat (Wilhelm *et al.* 2010), whereas the present study used granulocytes for LINE-1 and D4Z4 methylation quantification. Given DNA methylation patterns are cell specific, use of different white blood cells may give different results. Recent meta-analyses reported LINE-1 pyrosequencing assays are significantly heterogeneous between studies (Brennan & Flanagan 2012, Woo & Kim 2012). The sample size may account for observed differences. The sample size in the present study is almost twice as big as the other two studies. Alternatively, the possibility of chance finding cannot be ruled out and hence the results should be interpreted with caution.

Higher level of D4Z4 methylation was directly associated with increased UCB risk. This result is supported by findings of studies showing that bladder tumor and bladder cancer cell lines have elevated levels of D4Z4 methylation compared to the adjacent normal tissue (Choi *et al.* 2009, Cheng *et al.* 2004). D4Z4 repeat units contain the *DUX4* gene, a germline transcription factor, which is implicated in regulating genes involved in apoptosis, immune system regulation, and carcinogenesis (Geng *et al.* 2012), and hypermethylation of D4Z4 has been associated with repression of this gene in tumor tissues (Katargin *et al.* 2009). One of the target genes for *DUX4* is paired-like homeodomain 1 (*PITX1*) (Dixit *et al.* 2007), a tumor suppressor gene that regulates *TP53*, *RAS*, and *TERT* genes (Kolfschoten *et al.* 2005, Liu & Lobie 2007, Qi *et al.* 2011). *TP53* and *RAS* genes are frequently altered in UCB. This indicates that the presently observed association between increased risk and elevated D4Z4 methylation could be related to the deregulation of *DUX4* gene and the subsequent downstream effects. An alternative explanation is

that D4Z4 methylation could result in higher-order chromatin structural change with a positional effect. The association of both LINE-1 and D4Z4 with risk of UCB was modified by one-carbon metabolism genes *PEMT* and *FOLH1*, respectively. This highlights the importance of the one-carbon metabolism pathway in the modulation UCB cancer risk. Further epidemiological and experimental investigations are warranted to clarify the underlying mechanism behind these associations.

The association between LINE-1 and D4Z4 methylation in peripheral blood cells and the UCB outcomes has not been studied before. This study was the largest with longer follow-up time and detailed clinicopathological and molecular data to explore the prognostic value of genomic DNA methylation in granulocyte DNA. Both LINE-1 and D4Z4 methylation levels modeled showed no significant association with individual outcome in NMIBC (recurrence, progression and relapse) and MIBC (progression, bladder cancer-specific mortality and overall mortality) in both unadjusted and adjusted Cox proportional hazards models. Similar nonsignificant associations were observed in stratified analysis according to treatment, selected risk factors of UCB, and subphenotype analyses according to *FGFR3* mutation status and expression levels. A single study in 96 bladder cancer tissues reported an inverse association between LINE-1 methylation and recurrence and disease-specific survival time in the unadjusted model (Neuhausen *et al.* 2006). Further study is needed to evaluate the prognostic potential of genomic DNA methylation.

7.3. Strengths and limitations of this study

Limitations of this study are related to the nature of the study design, timing of baseline and exposure data, and blood sample collections, selection and misclassification biases. The SBC/EPICURO study was a multi-center hospital-based case-control study conducted in five regions of Spain (Asturias, Barcelona, Elche, Tenerife, and Valles). Therefore the distribution of exposures such as smoking habits among controls might not be representative of the source population in these five regions. Sociodemographic characteristics and tobacco smoking data were collected retrospectively through interview using risk factor questionnaires and the response rates for these questionnaires were 84 and 88% for cases and controls, respectively (Samanic *et al.* 2006). Although these response rates were higher, the possibility of selection bias due to

differences between participants and non-participants cannot be excluded. Also during the interview process the possibility of recall bias cannot be ruled out for both cases and controls. But because each case-control pair was recruited and matched from the same non-referral hospital, substantial selection and differential recall biases are not expected.

Blood sample used for DNA methylation determination was collected following admission to hospital but before treatment were started. DNA methylation is a stable yet dynamic epigenetic modification and hence it might be influenced by the disease process as well as hospital stay. The observed associations in this study might be the result of reverse causation. However, a prospective study that evaluated the association between DNA methylation in repetitive sequences and cancer risk reported that altered global DNA methylation was significantly associated risk of cancer (Zhu *et al.* 2011) indicating changes in DNA methylation preceded the disease process. Besides that, in the present project the diagnoses of the control subjects were not associated with both LINE-1 and D4Z4 methylation levels. Another potential problem is not all study participants had enough DNA to quantify genomic methylation. However, comparing those participants with and without DNA methylation data showed no substantial difference in the distribution of matching variables and smoking status (see Chapter III and Chapter V). Another limitation is the age and gender distribution of the control subjects used for the assessment of potential predictors of D4Z4 and LINE-1 methylation. Most of these subjects were older and predominantly males. This might limit the external validity of the result on DNA methylation predictors and therefore it should only apply for this demographic group.

Regarding data on genomic DNA methylation, methylation levels at four LINE-1 CpG sites from granulocytes were used as a surrogate for genome wide DNA methylation content. Methylation levels at CpG sites located in other genomic regions and repetitive DNA sequences such as *Alu* could also be used. However, only LINE-1 elements were validated as a surrogate for global DNA methylation levels (Estecio *et al.* 2007, Yang *et al.* 2004). Methylation level at six CpG sites in D4Z4 tandem repeats was also used as an additional marker in this study to evaluate macrosatellite methylation levels and UCB risk and progression. The levels of methylation in granulocytes might or might not differ from that of urothelial cells. Nonetheless methylation in granulocytes can be used as a biomarker of UCB risk and prognosis.

Usual dietary intakes of B vitamins, protein and fruits and vegetables were estimated from a comprehensive food frequency questionnaire and therefore it might not reflect the absolute content of each nutrient. Additionally not all the study participants returned the questionnaire but there was no significant difference in characteristics of those who had and had not completed the questionnaires (Garcia-Closas *et al.* 2007b). The smaller number of females in this study and missing data for dietary intakes, trace elements, *FGFR3* mutation and expression levels and might have limited the analyses for these variables due to reduced sample size.

Strengths of this work include its sample size, availability of diverse and reliable data on exposure status, genotype, clinicopatholical and molecular characteristics of tumor tissues. The SBC/EPICURO is largest study to date to evaluate the effects of genomic DNA methylation on UCB risk and survival. Bisulfite modification of DNA is the gold standard before measuring DNA methylation. LINE-1 and D4Z4 methylation levels were quantified by using pyrosequencing which is reliable method that provides accurate measurement (Lara *et al.* 2011, Laird 2010, Tost & Gut 2007). The within-sample coefficient of variations for LINE-1 and D4Z4 were 4.53 and 0.88%, respectively. All UCB patients included in the study were incident cases. Diagnosis, tumor stage and grade were confirmed by a panel of expert pathologists. Trained nurses collected data on vital status through direct telephone interviews the individual patient or their relatives, and UCB outcomes and treatments of UCB were retrieved from patient's hospital charts. Reliable information on trace elements were collected which was evidenced by their lower within-sample coefficient of variation (Amaral *et al.* 2012c). Genotypes with call rates of more than 95% were included in this analysis and a stringent cutoff value of p-value >0.05 was used to indicate departure from Hardy-Weinberg equilibrium.

7.4. Clinical and public health genomics implications

DNA methylation is a potentially reversible epigenetic modification. In this thesis, some factors potentially modifiable through medical interventions were associated with both LINE-1 and D4Z4 methylation levels. LINE-1 and D4Z4 methylation levels were associated with risk of UCB; particularly D4Z4 methylation was associated with a specific UCB subphenotype with distinct clinicopathological and molecular features. These results may have clinical and public health implications. One implication is to target DNA methylation using methylation modifying

drugs in individuals with specific exposure profile (for example arsenic exposed individuals) to mitigate the consequence of the exposure. Another benefit to public health genomics is that DNA methylation can be used to identify specific group individuals at high risk of onset of a certain disease or benefit from a specific preventive or therapeutic intervention. These approaches not only help realize targeted interventions also save time and recourses.

However, considering the level of evidence of an individual case-control study, further independent larger prospective cohort studies or preventive trials are required to provide accurate risk estimates to help harness the potential of DNA methylation in the clinical and public health settings.

7.5. Future directions

This thesis has identified novel predictors of genomic DNA methylation. In addition, it showed that genomic DNA methylation at was associated with increased risk of UCB. To identify additional factors that influence genomic DNA methylation, further studies with diverse internal and external exposure data are needed. Experimental studies in model systems will clarify the molecular mechanisms how these factors modulate DNA methylation and affect health and disease. To ascertain causality between DNA methylation and UCB risk and also enable subgroup analysis, well powered population based prospective studies using pre- and post-diagnosis collected DNA are required. Applying methods such as microarrays or single-base resolution bisulfite sequencing measure genome-wide methylation (Jones 2012) to identify novel CpG positions associated with UCB risk and progression are desirable. But until new studies recruit sufficient study subjects, establishing international collaboration between existing studies to enable detailed subgroup analyses, and identify novel DNA methylation*environment and DNA methylation*gene interactions is recommended.

Recently 5-methylcytocine has been shown to undergo sequential oxidative reaction catalyzed by iron(II)- and α-ketoglutarate-dependent TET family of hydroxylases to generate 5-hydroxymethylcytosine, 5-formylcytosine, and finally 5-carboxycytosine (Tahiliani *et al.* 2009, Ito *et al.* 2011). 5-hydroxymethylcytosine was found in considerable amount in LINE-1 sequences (Booth *et al.* 2012), and significantly globally reduced levels, compared to the matched normal tissues, have been reported in various human cancer types (lung, prostate, liver,

pancreatic, and breast cancers) (Yang *et al.* 2013). Therefore, it will be interesting to investigate how 5-hydroxymethylcytosine and the other cytosine modifications associate with UCB risk, progression as well as with 5-methylcytosine itself.

Finally, integrating DNA methylation with other 'omics' data would greatly improve our understanding of UCB, improve clinical outcomes, and help design novel preventive strategies to eventually reduce morbidity and mortality from the disease and improve quality of life.

CHAPTER VIII. CONCLUSIONS

Based on the results from this study, I conclude that:

I. Gender, tobacco type, arsenic, iron, nickel, and common genetic variants in the one-carbon metabolism pathway genes *DNMT3A*, *AS3MT*, *MTHFS*, *SLC19A1*, and *TCN2*, are independent predictors of LINE-1 methylation levels.

II. Gender, tobacco type, and selenium are independent predictors of D4Z4 methylation levels.

III. LINE-1 methylation levels are non-linearly associated with urothelial carcinoma of the bladder (UCB), i.e. both low and high levels of LINE-1 methylation conferred an increased risk for the disease.

IV. Five single nucleotide polymorphisms in *PEMT* genes may modify the association between LINE-1 methylation and risk of UCB.

V. High D4Z4 methylation levels increased the risk of developing low-grade non-muscle invasive bladder cancer overexpressing FGFR3.

VI. The effect of D4Z4 methylation is modified by levels of iron, nickel, and manganese. *FLOH1*-rs11040387 may also modify the risk of UCB conferred by D4Z4 methylation.

VII. LINE-1 and D4Z4 methylation levels had no prognostic value on recurrence, progression and survival in patients with non-muscle invasive and muscle invasive bladder cancer.

CONCLUSIONES

Basándonos en los resultados del presente estudio, concluyo que:

I. El género, el tipo de tabaco, el arsénico, el hierro, el níquel, y variantes genéticas comunes en genes de la vía del metabolismo del monocarbono (*DNMT3A*, *AS3MT*, *MTHFS*, *SLC19A1* y *TCN2*) son predictores independientes de los niveles de metilación de LINE-1.

II. El género, el tipo de tabaco y el selenio son predictores independientes de los niveles de metilación de D4Z4.

III. Los niveles de metilación de LINE-1 están asociados con el carcinoma uroterial de vejiga (CUV) de manera no lineal, de tal modo que niveles de baja y alta metilación en LINE-1 confieren un aumento del riesgo de padecer la enfermedad.

IV. Cinco polimorfismos de nucleótido simple del gen *PEMT* modifican modificar la asociación entre la metilación de LINE-1 y el riesgo de desarrollar CUV.

V. Niveles elevados de metilación de D4Z4 incrementan el riesgo de desarrollar cáncer de vejiga no-invasivo del músculo de bajo grado con sobreexpresión de FGFR3.

VI. El efecto de la metilación de D4Z4 es modificado por los niveles de hierro, níquel y manganeso. *FLOH1*-rs11040387 también modificar la susceptibilidad al CUV conferida por la metilación de D4Z4.

VII. Los niveles de metilación de LINE-1 y D4Z4 no tienen valor pronóstico independiente de recurrencia, progresión y supervivencia tanto en pacientes con cáncer de vejiga no-invasivo como invasivo del músculo.

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APPENDIX

SPANISH BLADDER CANCER/EPICURO STUDY INVESTIGATORS

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SUPPLEMENTARY MATERIALS

SUPPLEMENTARY MATERIALS CHAPTER III

Supplementary Table S3-1 List of genes involved in the one-carbon metabolism pathway selected for the present study

Chromosome location	Gene symbol [ID]*	Gene name	Gene function
1p31.1	*CTH*	Cystathionase	Converts cystathione derived from methionine into cysteine.
1p36.3	*MTHFR*	Methylenetetrahydrofolate reductase (NAD(P)H)	Catalyzes the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate.
1q43	*MTR*	5-methyltetrahydrofolate-homocysteine methyltransferase	Catalyzes the final step in methionine biosynthesis.
2p23	*DNMT3A*	DNA (cytosine-5-)-methyltransferase 3 alpha	Functions in de novo methylation of cytosine residues.
2q35	*ATIC*	5-aminoimidazole-4-carboxamide ribonucleotide formyltransferase/IMP(inosine monophosphate) cyclohydrolase	Catalyzes the last two steps of the de novo purine biosynthetic pathway. The N-terminal domain has phosphoribosylaminoimidazolecarboxamide formyltransferase activity, and the C-terminal domain has IMP cyclohydrolase activity.
3p21.1	*CHDH*	Choline dehydrogenase	Encodes a protein that localizes to the mitochondrion. Variations in this gene can affect susceptibility to choline deficiency.
3q21.3	*ALDH1L1*	Aldehyde dehydrogenase 1 family, member L1	Catalyzes the conversion of 10-formyltetrahydrofolate, NADP, and water to tetrahydrofolate, NADPH, and carbon dioxide.
5p15.31	*MTRR*	5-methyltetrahydrofolate-homocysteine methyltransferase reductase	Regenerates a functional methionine synthase via reductive methylation
5q11.2-q13.2	*DHFR*	Dihydrofolate reductase	Converts dihydrofolate into tetrahydrofolate, a methyl group shuttle required for the de novo synthesis of purines, thymidylic acid, and certain amino acids.
5q13.1-q15	*BHMT*	Betaine-homocysteine S-methyltransferase	Catalyzes the conversion of betaine and homocysteine to dimethylglycine and methionine, respectively.
6p12	*GNMT*	Glycine N-methyltransferase	Catalyzes the conversion of S-adenosyl-L-methionine (along with glycine) to S-adenosyl-L-homocysteine and sarcosine.

Supplementary Table S3-1 (cont.) List of genes involved in the one-carbon metabolism pathway selected for the present study

Chromosome location	Gene symbol [ID]*	Gene name	Gene function
10q24.32	*AS3MT*	Arsenic (+3 oxidation state) methyltransferase	Catalyzes the transfer of a methyl group from S-adenosyl-L-methionine to trivalent arsenical and play a role in arsenic metabolism.
11p11.2	*FOLH1*	Folate hydrolase (prostate-specific membrane antigen)1/glutamate carboxypeptidase II/	Acts as a glutamate carboxypeptidase on different alternative substrates, including the nutrient folate and the neuropeptide N-acetyl-l-aspartyl-l-glutamate.
14q24	*MTHFD*	Methylenetetrahydrofolate dehydrogenase (NADP+ dependent) 1	Catalyzes one of three sequential reactions in the interconversion of 1-carbon derivatives of tetrahydrofolate.
15q25.1	*MTHFS*	5,10-methenyltetrahydrofolate synthetase	Catalyzes the conversion of 5-formyltetrahydrofolate to 5,10-methenyltetrahydrofolate, a precursor of reduced folates involved in 1-carbon metabolism.
17p11.2	*PEMT*	Phosphatidylethanolamine N-methyltransferase	Converts phosphatidylethanolamine to phosphatidylcholine by sequential methylation in the liver.
17p11.2	*SHMT1*	Serine hydroxymethyltransferase 1	Catalyzes the reversible conversion of serine and tetrahydrofolate to glycine and 5,10-methylene tetrahydrofolate. This reaction provides one carbon units for synthesis of methionine, thymidylate, and purines in the cytoplasm.
18p11.32	*TYMS*	Thymidylate synthetase	Catalyzes the methylation of deoxyuridylate to deoxythymidylate using 5,10-methylenetetrahydrofolate as a cofactor.
19p13.2	*DNMT1*	DNA (cytosine-5-)-methyltransferase 1	Establishes and regulates tissue-specific patterns of methylated cytosine residues.
20q11.2	*DNMT3B*	DNA (cytosine-5-)-methyltransferase 3 beta	Functions in de novo methylation of cytosine residues.
21q22.1; 21q22.11	*GART*	Glycinamide ribonucleotide transformylase	Encodes a protein that has phosphoribosylglycinamide formyltransferase, phosphoribosylglycinamide synthetase, phosphoribosylaminoimidazole synthetase activity which is required for de novo purine biosynthesis.

Supplementary Table S3-1 (cont.) List of genes involved in the one-carbon metabolism pathway selected for the present study

Chromosome location	Gene symbol [ID]*	Gene name	Gene function
21q22.3	*CBS*	Cystathionine-beta-synthase	Acts as a homotetramer to catalyze the conversion of homocysteine to cystathionine, the first step in the transsulfuration pathway.
21q22.3	*SLC19A1*	Solute carrier family 19 (folate transporter), member 1	Transports folate and is involved in the regulation of intracellular concentrations of folate.
22q12.2	*TCN2*	Transcobalamin II	Binds and mediates the transport of vitamin B_{12} into cells.

*Maglott D, Ostell J, Pruitt KD, Tatusova T. 2005. Entrez Gene: gene-centered information at NCBI. Nucleic Acids Res 33:D54-D58.

Supplementary Table S3-2 Distribution of SNPs studied in the present study by genotyping platform

Genotyping Assay	Gene Symbol	dbSNP ID	Gene Symbol	dbSNP ID
Illumina Infinium® Human1M-Duo				
	AS3MT	rs4532960	*ATIC*	rs3821353
	AS3MT	rs10509760	*ATIC*	rs16853782
	AS3MT	rs1046778	*ATIC*	rs1464864
	AS3MT	rs7085854	*ATIC*	rs7563206
	AS3MT	rs10748835	*ATIC*	rs4673981
	AS3MT	rs3740392	*ATIC*	rs4531931
	AS3MT	rs7085104	*ATIC*	rs6750194
	AS3MT	rs11191439	*ATIC*	rs6760069
	AS3MT	rs3740394	*CHDH*	rs920253
	AS3MT	rs11191457	*CHDH*	rs6801605
	AS3MT	rs12416687	*CHDH*	rs13317328
	ATIC	rs4610054	*CHDH*	rs3755817
	ATIC	rs10165919	*CHDH*	rs9001
	ATIC	rs12997662	*CHDH*	rs6445606
	ATIC	rs1404772	*CHDH*	rs3774616
	ATIC	rs11683778	*CHDH*	rs2289209
	ATIC	rs10498034	*CHDH*	rs9835128
	ATIC	rs6706415	*CHDH*	rs2241807
	ATIC	rs4673993	*CHDH*	rs9836592
	ATIC	rs10498036	*DNMT1*	rs2288349
	ATIC	rs3772078	*DNMT1*	rs2288350
	ATIC	rs4476347	*DNMT1*	rs8111085
	ATIC	rs7604984	*DNMT1*	rs2162560
	ATIC	rs11892429	*DNMT1*	rs4804125
	ATIC	rs6757920	*DNMT1*	rs11672909
	ATIC	rs4369857	*DNMT1*	rs6511677
	ATIC	rs11687225	*DNMT1*	rs11085720
	ATIC	rs16853834	*DNMT1*	rs7253062
	ATIC	rs1880586	*DNMT1*	rs35918857
	ATIC	rs2372535	*DNMT1*	rs4804122
	ATIC	rs1983462	*DNMT1*	rs17291414
	ATIC	rs12614943	*DNMT1*	rs4804494
	ATIC	rs10179873	*DNMT1*	rs9305012
	ATIC	rs4673965	*DNMT1*	rs16999714
	ATIC	rs10932605	*DNMT1*	rs2116940
	ATIC	rs9789571	*DNMT1*	rs8101626
	ATIC	rs10197653	*DNMT1*	rs10418707
	ATIC	rs1808119	*DNMT1*	rs8112895
	ATIC	rs4673991	*DNMT1*	rs10420338
	ATIC	rs7604425	*DNMT1*	rs12462004
	ATIC	rs4672768	*DNMT1*	rs4804490
	ATIC	rs16853826	*DNMT1*	rs2290684
	ATIC	rs10197559	*DNMT1*	rs2228611
	ATIC	rs1404774	*DNMT1*	rs759920

Supplementary Table S3-2 (cont.)

Genotyping Assay	Gene Symbol	dbSNP ID	Gene Symbol	dbSNP ID
	DNMT3A	rs6722613	*DNMT3B*	rs4911107
	DNMT3A	rs7581217	*DNMT3B*	rs910085
	DNMT3A	rs7575625	*DNMT3B*	rs6058891
	DNMT3A	rs1465825	*DNMT3B*	rs2424932
	DNMT3A	rs12987326	*DNMT3A*	rs12999687
	DNMT3A	rs734693	*DNMT3B*	rs6058893
	DNMT3A	rs13401241	*DNMT3B*	rs6087990
	DNMT3A	rs11694842	*DNMT3B*	rs6058897
	DNMT3A	rs7583409	*DNMT3B*	rs4911263
	DNMT3A	rs13427202	*DNMT3B*	rs2424928
	DNMT3A	rs11887120	*DNMT3B*	rs4911108
	DNMT3A	rs12995968	*DNMT3B*	rs6141813
	DNMT3A	rs13002567	*DNMT3B*	rs6058896
	DNMT3A	rs10460566	*DNMT3B*	rs6058883
	DNMT3A	rs6711622	*DNMT3B*	rs853858
	DNMT3A	rs13036246	*DNMT3B*	rs6119286
	DNMT3A	rs749130	*DNMT3B*	rs437302
	DNMT3A	rs17745484	*DNMT3B*	rs2424921
	DNMT3A	rs1550117	*FOLH1*	rs7102702
	DNMT3A	rs6749992	*FOLH1*	rs1819409
	DNMT3A	rs7586294	*FOLH1*	rs11040432
	DNMT3A	rs11892646	*FOLH1*	rs11040387
	DNMT3A	rs11677670	*FOLH1*	rs12098986
	DNMT3A	rs11683424	*FOLH1*	rs202712
	DNMT3A	rs7560488	*FOLH1*	rs1814175
	DNMT3A	rs7587636	*FOLH1*	rs3862350
	DNMT3A	rs11681447	*FOLH1*	rs679470
	DNMT3A	rs6713377	*FOLH1*	rs7929543
	DNMT3A	rs2304429	*FOLH1*	rs2696935
	DNMT3A	rs2289093	*FOLH1*	rs7117247
	DNMT3A	rs34048824	*FOLH1*	rs11040261
	DNMT3A	rs7594432	*FOLH1*	rs10769558
	DNMT3A	rs7605753	*FOLH1*	rs1917321
	DNMT3A	rs1369703	*FOLH1*	rs4144700
	DNMT3B	rs6119285	*FOLH1*	rs7358352
	DNMT3B	rs2424913	*FOLH1*	rs588458
	DNMT3B	rs2424908	*FOLH1*	rs7113251
	DNMT3B	rs2424914	*FOLH1*	rs3862342
	DNMT3B	rs6058894	*FOLH1*	rs17556442
	DNMT3B	rs6057645	*FOLH1*	rs10839229
	DNMT3B	rs6087988	*FOLH1*	rs11040390
	DNMT3B	rs6058869	*FOLH1*	rs4094478
	DNMT3B	rs709046	*FOLH1*	rs12293923
	DNMT3B	rs2424922	*FOLH1*	rs7951180
	DNMT3B	rs2424906	*FOLH1*	rs1164685

Supplementary Table S3-2 (cont.)

Genotyping Assay	Gene Symbol	dbSNP ID	Gene Symbol	dbSNP ID
	FOLH1	rs10839295	*FOLH1*	rs11040421
	FOLH1	rs10839296	*FOLH1*	rs12222221
	FOLH1	rs10839224	*FOLH1*	rs1847638
	FOLH1	rs16906158	*FOLH1*	rs1917311
	FOLH1	rs10839210	*GART*	rs2834235
	FOLH1	rs7120743	*GART*	rs4817577
	FOLH1	rs10501325	*GART*	rs6517178
	FOLH1	rs3960835	*GART*	rs7283354
	FOLH1	rs7113075	*GART*	rs4817580
	FOLH1	rs2696923	*GART*	rs2834233
	FOLH1	rs11040263	*GART*	rs12482067
	FOLH1	rs683680	*GART*	rs8971
	FOLH1	rs10400277	*GART*	rs2834232
	FOLH1	rs202718	*GART*	rs2154583
	FOLH1	rs34033751	*GART*	rs4817579
	FOLH1	rs11040353	*GART*	rs2834231
	FOLH1	rs7111215	*GNMT*	rs3800292
	FOLH1	rs648372	*GNMT*	rs4987173
	FOLH1	rs7111711	*GNMT*	rs10948059
	FOLH1	rs650826	*GNMT*	rs7759302
	FOLH1	rs7926211	*GNMT*	rs9462856
	FOLH1	rs11607791	*MTHFD1*	rs2281603
	FOLH1	rs1164681	*MTHFD1*	rs7144437
	FOLH1	rs12797843	*MTHFD1*	rs17101854
	FOLH1	rs12797853	*MTHFD1*	rs3783728
	FOLH1	rs7124266	*MTHFD1*	rs2983733
	FOLH1	rs632220	*MTHFD1*	rs4902278
	FOLH1	rs202700	*MTHFD1*	rs11627387
	FOLH1	rs202676	*MTHFD1*	rs1256095
	FOLH1	rs1809986	*MTHFD1*	rs8019804
	FOLH1	rs11040416	*MTHFD1*	rs1256114
	FOLH1	rs7117025	*MTHFD1*	rs9323450
	FOLH1	rs7107178	*MTHFD1*	rs2983736
	FOLH1	rs7934591	*MTHFD1*	rs11629135
	FOLH1	rs10839239	*MTHFD1*	rs2987981
	FOLH1	rs598841	*MTHFD1*	rs1076991
	FOLH1	rs2865908	*MTHFD1*	rs1956545
	FOLH1	rs1846285	*MTHFD1*	rs3818239
	FOLH1	rs4441015	*MTHFD1*	rs11627525
	FOLH1	rs11040106	*MTHFD1*	rs17751556
	FOLH1	rs2866358	*MTHFD1*	rs2987969
	FOLH1	rs17728676	*MTHFD1*	rs1256107
	FOLH1	rs6485991	*MTHFD1*	rs1256112
	FOLH1	rs660439	*MTHFD1*	rs10498514
	FOLH1	rs7102641	*MTHFD1*	rs11158542
	FOLH1	rs4495895	*MTHFD1*	rs8015278

Supplementary Table S3-2 (cont.)

Genotyping Assay	Gene Symbol	dbSNP ID	Gene Symbol	dbSNP ID
	MTHFD1	rs1950902	*MTHFS*	rs4779141
	MTHFD1	rs2236222	*MTHFS*	rs17211644
	MTHFD1	rs35020344	*MTHFS*	rs1870576
	MTHFD1	rs6573559	*MTHFS*	rs17209637
	MTHFD1	rs12884767	*MTHFS*	rs1077965
	MTHFD1	rs2236225	*MTHFS*	rs3893384
	MTHFD1	rs2295639	*MTHFS*	rs6495441
	MTHFD1	rs8011839	*MTHFS*	rs4778721
	MTHFD1	rs2236224	*MTHFS*	rs282795
	MTHFD1	rs1256142	*MTHFS*	rs282778
	MTHFD1	rs8003379	*MTHFS*	rs898436
	MTHFD1	rs17824591	*MTHFS*	rs16971249
	MTHFD1	rs8003567	*MTHFS*	rs12898670
	MTHFD1	rs11158540	*MTHFS*	rs2733103
	MTHFD1	rs11158538	*MTHFS*	rs12913164
	MTHFD1	rs8018032	*MTHFS*	rs282792
	MTHFS	rs1473406	*MTHFS*	rs1604503
	MTHFS	rs204942	*MTHFS*	rs17285431
	MTHFS	rs2115536	*MTHFS*	rs17279885
	MTHFS	rs17279286	*MTHFS*	rs2586154
	MTHFS	rs582172	*MTHFS*	rs12592743
	MTHFS	rs11852515	*MTHFS*	rs435689
	MTHFS	rs376863	*MTHFS*	rs12903985
	MTHFS	rs1081231	*MTHFS*	rs4779148
	MTHFS	rs2035027	*MTHFS*	rs1081235
	MTHFS	rs4393531	*MTHFS*	rs600671
	MTHFS	rs7177659	*MTHFS*	rs17279753
	MTHFS	rs2586153	*MTHFS*	rs445263
	MTHFS	rs8041943	*MTHFS*	rs4778719
	MTHFS	rs282776	*MTHFS*	rs6495446
	MTHFS	rs282802	*MTHFS*	rs282787
	MTHFS	rs1880580	*MTHFS*	rs16971260
	MTHFS	rs16971253	*MTHFS*	rs16971450
	MTHFS	rs4779140	*MTHFS*	rs7174668
	MTHFS	rs7175620	*MTHFS*	rs685487
	MTHFS	rs4779165	*MTHFS*	rs12438477
	MTHFS	rs166868	*MTHFS*	rs10163099
	MTHFS	rs372447	*MTHFS*	rs12900076
	MTHFS	rs282772	*MTHFS*	rs12591436
	MTHFS	rs11855092	*MTHFS*	rs1074390
	MTHFS	rs12905663	*MTHFS*	rs282814
	MTHFS	rs3897953	*MTHFS*	rs443394
	MTHFS	rs16971231	*MTHFS*	rs12148881
	MTHFS	rs8923	*MTHFS*	rs12898642
	MTHFS	rs378057	*MTHFS*	rs12910340
	MTHFS	rs2586182	*MTHFS*	rs7177027

Supplementary Table S3-2 (cont.)

Genotyping Assay	Gene Symbol	dbSNP ID	Gene Symbol	dbSNP ID
	MTHFS	rs17284990	*PEMT*	rs3760188
	MTHFS	rs16971252	*SLC19A1*	rs2838969
	MTHFS	rs1380642	*SLC19A1*	rs9976878
	MTHFS	rs2115540	*SLC19A1*	rs2838970
	MTHFS	rs770144	*SLC19A1*	rs9977111
	MTHFS	rs2733106	*SLC19A1*	rs1051266
	MTHFS	rs1460177	*SLC19A1*	rs2183601
	MTHFS	rs8034036	*SLC19A1*	rs3788190
	PEMT	rs7217764	*SLC19A1*	rs12482346
	PEMT	rs748196	*SLC19A1*	rs767138
	PEMT	rs8074074	*SLC19A1*	rs8128681
	PEMT	rs7219568	*SLC19A1*	rs8129350
	PEMT	rs4244599	*SLC19A1*	rs8129445
	PEMT	rs9897362	*SLC19A1*	rs2838964
	PEMT	rs750546	*SLC19A1*	rs4819128
	PEMT	rs11869600	*SLC19A1*	rs8128676
	PEMT	rs4646404	*SLC19A1*	rs2150460
	PEMT	rs2124344	*SLC19A1*	rs9974061
	PEMT	rs4646341	*SLC19A1*	rs6518253
	PEMT	rs8081810	*SLC19A1*	rs914232
	PEMT	rs9910747	*SLC19A1*	rs2330183
	PEMT	rs4924922	*SLC19A1*	rs1023159
	PEMT	rs1020697	*SLC19A1*	rs944422
	PEMT	rs897453	*SLC19A1*	rs1888530
	PEMT	rs4646383	*SLC19A1*	rs4819138
	PEMT	rs11658944	*SLC19A1*	rs12373907
	PEMT	rs4646385	*SLC19A1*	rs2838958
	PEMT	rs1109859	*SLC19A1*	rs12627639
	PEMT	rs4646350	*SLC19A1*	rs914231
	PEMT	rs4646340	*SLC19A1*	rs2877078
	PEMT	rs4646359	*SLC19A1*	rs9306139
	PEMT	rs11871738	*SLC19A1*	rs2838965
	PEMT	rs11656215	*SLC19A1*	rs1888533
	PEMT	rs4646344	*SLC19A1*	rs1055345
	PEMT	rs3785499	*SLC19A1*	rs3177999
	PEMT	rs7946	*SLC19A1*	rs7279305
	PEMT	rs8068641	*SLC19A1*	rs7276295
	PEMT	rs4646364	*SLC19A1*	rs4818789
	PEMT	rs4925048	*TCN2*	rs16988828
	PEMT	rs4479310	*TCN2*	rs5749135
	PEMT	rs4646410	*TCN2*	rs740234
	PEMT	rs9890064	*TCN2*	rs1801198
	PEMT	rs12453139	*TCN2*	rs2267163
	PEMT	rs7215833	*TCN2*	rs5753231
	PEMT	rs7220132	*TCN2*	rs2283873
	PEMT	rs8074191	*TCN2*	rs4820887

Supplementary Table S3-2 (cont.)

Genotyping Assay	Gene Symbol	dbSNP ID	Gene Symbol	dbSNP ID
	TCN2	rs10418	*SLC19A1*	rs4434082
	TCN2	rs1131603	*SLC19A1*	rs11701960
	TCN2	rs5749131	*SLC19A1*	rs9980967
	TCN2	rs9606756	*SLC19A1*	rs2838973
	TCN2	rs2301955	*SLC19A1*	rs4819130
	TCN2	rs9621049	*SLC19A1*	rs914238
	TCN2	rs9306264	*SLC19A1*	rs2838977
	SLC19A1	rs3788200		
	SLC19A1	rs2838961		
	SLC19A1	rs8128050		
Illumina GoldenGate®				
	ALDH1L1	rs1127717	*TYMS*	rs1059394
	ALDH1L1	rs2305230		
	ALDH1L1	rs9282690		
	BHMT	rs567754		
	BHMT	rs617219		
	CBS	rs234706		
	CBS	rs12613		
	CBS	rs6586282		
	CTH	rs663465		
	CTH	rs663649		
	CTH	rs6413471		
	CTH	rs473334		
	CTH	rs515064		
	CTH	rs559062		
	DHFR	rs35709834		
	DHFR	rs865646		
	DHFR	rs1650697		
	MTHFD	rs1667627		
	MTHFR	MTHFR_02__2_i_order		
	MTHFR	rs1801133		
	MTHFR	rs2066470		
	MTHFR	rs12121543		
	MTR	MTR_01__2_i_order		
	MTR	rs1805087		
	MTR	rs2275565		
	MTR	rs2275566		
	MTRR	rs2287780		
	MTRR	rs9332		
	MTRR	rs10380		
	MTRR	rs1802059		
	MTRR	rs8659		
	MTRR	rs2287779		
	SLC19A1	rs1051298		
	TYMS	rs699517		
	TYMS	rs2790		

Supplementary Table S3-2 (cont.)

Genotyping Assay	Gene Symbol	dbSNP ID
TaqMan®		
	MTHFR	rs1801131
	MTHFR	MTHFR_02_ORDER
	MTR	MTR_01_ORDER
	MTRR	rs1801394
	SHMT1	rs1979277
	SHMT1	rs1979276
	SHMT1	rs3783
	SLC19A1	rs1051296

dbSNP ID, dbSNP identifier number.

Supplementary Table S3-3 List of single nucleotide polymorphisms with significant Fisher's exact test selected for further analysis (p-value ≤ 0.05)

dbSNP Identifier	N	MAF	Chromosome	Location*	Gene	Region in the gene	P-HWE	Fisher's exact test p-value†
rs11683424	875	0.12	2	25342636	*DNMT3A*	Intron	0.20	0.006
rs7581217	875	0.39	2	25378448	*DNMT3A*	Intron	0.62	0.002
rs1550117	875	0.08	2	25419411	*DNMT3A*	Flanking 5'UTR	0.81	0.009
rs4369857	875	0.04	2	215841917	*ATIC*	Flanking 5'UTR	1.00	0.02
rs7563206	874	0.47	2	215898899	*ATIC*	Intron	0.84	0.04
rs1880586	875	0.47	2	215903416	*ATIC*	Intron	0.89	0.04
rs4673991	875	0.32	2	215920334	*ATIC*	Intron	0.31	0.004
rs4673993	875	0.32	2	215920584	*ATIC*	Intron	0.31	0.004
rs4672768	873	0.32	2	215922369	*ATIC*	Intron	0.31	0.005
rs10498036	875	0.40	2	215922737	*ATIC*	Flanking 3'UTR	0.57	0.004
rs7604984	875	0.40	2	215925884	*ATIC*	Flanking 3'UTR	0.62	0.003
rs7085104	875	0.38	10	104618863	*AS3MT*	Flanking 5'UTR	1.00	0.02
rs1077965	875	0.41	15	77852392	*MTHFS*	Flanking 3'UTR	0.58	0.007
rs11855092	875	0.24	15	77877293	*MTHFS*	Flanking 3'UTR	0.31	0.03
rs1380642	875	0.18	15	77883926	*MTHFS*	Flanking 3'UTR	0.57	0.01
rs4646340	875	0.37	17	17434740	*PEMT*	Intron	0.51	0.04
rs7215833	875	0.36	17	17448033	*PEMT*	Flanking 5'UTR	0.51	0.02
rs914238	875	0.49	21	45840089	*SLC19A1*	Flanking 5'UTR	0.89	0.002
rs2838965	873	0.42	21	45846212	*SLC19A1*	Flanking 5'UTR	0.33	0.02
rs9606756	875	0.12	22	29336860	*TCN2*	Exon	0.87	0.005
rs9621049	875	0.11	22	29343419	*TCN2*	Exon	0.86	0.02
rs4820887	875	0.10	22	29346914	*TCN2*	Intron	0.85	0.01

MAF, minor allele frequency. P-HWE, Chi-square test p-value for Hardy-Weinberg equilibrium.
*Human Genome Build 36.3 location.
†Fisher's exact test p-value from comparison of categorical variables LINE-1 methylation in tertiles (<56.7%, 56.7–58.6%, and >58.6%) and SNPs modeled according to codominant mode of in heritance coded as "0" for wild type, "1" heterozygous variant, "2" for homozygous variant.

Supplementary Table S3-4 Distributional characteristics of study subjects and LINE-1 methylation in the SBC/EPICURO Study

Variables	N	Percent	LINE-1 methylation (%) Median (IQR)	P-value*
Age (years)				
<60	253	28.4	57.4 (3.7)	0.9
60-69	342	38.3	57.4 (3.2)	
70+	297	33.3	57.5 (3.2)	
Gender				
Male	792	88.8	57.5 (3.3)	0.04
Female	100	11.2	57.1 (3.2)	
Region				
Barcelona	168	18.8	57.5 (3.0)	0.1
Vallès	135	15.1	57.1 (2.6)	
Elche	73	8.2	57.1 (2.5)	
Tenerife	145	16.3	57.3 (3.2)	
Asturias	371	41.6	57.7 (3.9)	
Body mass index (kg/m^2)				
<25.0	372	53.4	57.4 (3.3)	0.9
25.0-26.99	148	21.2	57.4 (3.7)	
27.0-29.99	120	17.2	57.5 (3.2)	
≥30.0	57	8.2	57.6 (3.5)	
Missing data	195			
Smoking status				
Non-smoker	255	28.7	57.4 (3.2)	0.2
Occasional smoker	66	7.4	58.0 (3.3)	
Former smoker	329	37.0	57.4 (3.3)	
Current smoker	239	26.9	57.4 (3.7)	
Missing data	3			
Tobacco type				
Non-smoker	255	31.0	57.4 (3.2)	0.2
Blond only	99	12.0	57.0 (3.2)	
Black only	219	26.5	57.7 (3.6)	
Blond and black	154	18.7	57.3 (3.6)	
Unknown	97	11.8	57.7 (3.0)	
Missing data	68			
Controls' diagnosis				
Hernia	332	37.2	57.4 (3.1)	0.8
Fracture & Trauma	263	29.5	57.5 (4.0)	
Hydrocele	122	13.7	57.6 (3.3)	
Other Abdominal Surgery	99	11.1	57.2 (2.9)	

Supplementary Table S3-4 (cont.) Distributional characteristics of study subjects and LINE-1 methylation in the SBC/EPICURO Study

Variables	N	Percent	LINE-1 methylation (%) Median (IQR)	P-value*
Other Diseases	76	8.5	57.6 (3.8)	
Vitamin B_1 intake (µg/day/kcal)				
< 0.66	323	50.1	57.5 (3.8)	0.4
≥0.66	322	49.9	57.4 (3.3)	
Missing data	247			
Vitamin B_2 intake (µg/day/kcal)				
< 0.91	323	50.1	57.4 (3.7)	0.9
≥0.91	322	49.9	57.5 (3.6)	
Missing Data	247			
Vitamin B_3 intake (µg/day/kcal)				
<10.08	323	50.1	57.5 (3.9)	0.5
≥10.08	322	49.9	57.4 (3.3)	
Missing Data	247			
Vitamin B_6 intake (µg/day/kcal)				
<1.00	323	50.1	57.4 (3.9)	0.5
≥1.00	322	49.9	57.4 (3.4)	
Missing Data	247			
Vitamin B_{12} intake (µg/day/kcal)				
<3.98	323	50.1	57.4 (3.7)	0.8
≥3.98	322	49.9	57.4 (3.6)	
Missing Data	247			
Folate intake (µg/day/kcal)				
<166.74	323	50.1	57.4 (3.9)	0.5
≥166.74	322	49.9	57.5 (3.3)	
Missing Data	247			
Protein intake (µg/day/kcal)				
<46.68	323	50.1	57.4 (3.7)	0.97
≥4.33	322	49.9	57.4 (3.6)	
Missing Data	247			
Alcohol intake (µg/day/kcal)				
<4.33	323	50.1	57.4 (3.6)	0.4
≥4.33	322	49.9	57.4 (3.8)	
Missing Data	247			
Fruit intake (g/day/kcal)				
<172.71	320	50.1	57.3 (3.2)	0.6
≥172.71	319	49.9	57.6 (4.2)	
Missing Data	253			

Supplementary Table S3-4 (cont.) Distributional characteristics of study subjects and LINE-1 methylation in the SBC/EPICURO Study

Variables	N	Percent	LINE-1 methylation (%) Median (IQR)	P-value*
Vegetable intake (g/day/kcal)				
<107.12	320	50.0	57.3 (4.0)	0.3
≥107.12	320	50.0	57.6 (3.3)	
Missing Data	252			
Fruit and vegetable intake (g/day/kcal)				
<295.61	320	50.1	57.4 (3.5)	0.9
≥295.61	319	49.9	57.4 (3.9)	
Missing Data	253			
Toenail aluminum (µg/g)				
< 10.51	329	50.0	57.5 (3.6)	0.5
≥ 10.51	329	50.0	57.4 (3.3)	
Missing Data	234			
Toenail arsenic (µg/g)				
<0.07	330	50.1	57.5 (3.8)	0.7
≥0.07	329	49.9	57.4 (3.3)	
Missing Data	233			
Toenail cadmium (µg/g)				
<0.01	330	50.1	57.5 (3.2)	0.4
≥0.01	329	49.9	57.4 (3.6)	
Missing Data	233			
Toenail chromium (µg/g)				
< 0.41	330	50.1	57.5 (3.8)	0.6
≥ 0.41	329	49.9	57.4 (3.2)	
Missing Data	233			
Toenail copper (µg/g)				
< 3.38	329	49.9	57.5 (3.3)	0.9
≥3.38	330	50.1	57.4 (3.5)	
Missing Data	233			
Toenail iron (µg/g)				
< 14.61	329	50.0	57.4 (3.1)	0.3
≥ 14.61	329	50.0	57.6 (3.7)	
Missing Data	234			
Toenail lead (µg/g)				
<0.40	329	50.1	57.5 (3.1)	0.7
≥0.40	330	49.9	57.4 (3.6)	
Missing Data	233			

Supplementary Table S3-4 (cont.) Distributional characteristics of study subjects and LINE-1 methylation in the SBC/EPICURO Study

Variables	N	Percent	LINE-1 methylation (%) Median (IQR)	P-value*
Toenail manganese (µg/g)				
< 0.33	330	50.1	57.4 (3.3)	0.9
≥ 0.33	329	49.9	57.5 (3.6)	
Missing Data	233			
Toenail nickel (µg/g)				
< 0.47	329	49.9	57.5 (3.0)	0.8
≥ 0.47	330	50.1	57.4 (3.9)	
Missing Data	233			
Toenail selenium (µg/g)				
<0.56	330	50.1	57.4 (3.4)	0.6
≥0.56	329	49.9	57.5 (3.5)	
Missing Data	233			
Toenail vanadium (µg/g)				
< 0.02	326	50.1	57.5 (3.7)	0.6
≥ 0.02	325	49.9	57.4 (3.3)	
Missing Data	241			
Toenail zinc (µg/g)				
< 103.20	329	49.9	57.5 (3.3)	0.8
≥103.20	330	50.1	57.4 (3.7)	
Missing data	233			
***NAT2* phenotype**				
Rapid/Intermediate acetylator	389	43.9	57.4 (3.5)	0.8
Slow acetylator	498	56.1	57.5 (3.2)	
Missing data	5			
***GSTM1* genotype**				
(+/+, +/-)	421	47.7	57.4 (3.5)	0.6
(-/-)	462	52.3	57.5 (3.1)	
Missing data	9			
***GSTT1* genotype**				
(+/+, +/-)	688	77.7	57.5 (3.3)	0.2
(-/-)	198	22.3	57.4 (3.3)	
Missing data	6			

IQR, interquartile range.
*P-value from the Kruskal-Wallis test.

Supplementary Table S3-5 Association between LINE-1 methylation and individual characteristics of study subjects in the SBC/EPICURO Study

Variables	N	β (95% CI)*	P-value
Age (years)	892	-0.004 (-0.02, 0.01)	0.6
Gender			
Male	792	Ref	
Female	100	-0.5 (-1.0, 0.04)	0.07
Region			
Barcelona	168	Ref	
Valles	135	-0.2 (-0.7, 0.4)	0.6
Elche	73	-0.5 (-1.1, 0.2)	0.2
Tenerife	145	-0.1 (-0.7, 0.4)	0.6
Asturias	371	0.2 (-0.3, 0.6)	0.5
Body mass index (kg/m^2)			
<25.0	372	Ref	
25.0-26.99	148	-0.1 (-0.6, 0.3)	0.5
27.0-29.99	120	0.2 (-0.3, 0.7)	0.3
≥30.0	57	0.007 (-0.7, 0.7)	0.9
Missing data	195		
Controls' diagnosis			
Hernia	332	Ref	
Fracture & Trauma	263	-0.04 (-0.5, 0.4)	0.8
Hydrocele	122	0.2 (-0.4, 0.7)	0.5
Other Abdominal Surgery	99	0.1 (-0.7, 0.4)	0.6
Other Diseases	76	0.2 (-0.4, 0.8)	0.6
Dietary intake†			
Vitamin B_1 (µg/day/kcal)	645	0.6 (-0.5, 1.8)	0.3
Vitamin B_2 (µg/day/kcal)	645	0.2 (-0.5, 0.9)	0.6
Vitamin B_3 (µg/day/kcal)	645	0.03 (-0.04, 0.1)	0.4
Vitamin B_6 (µg/day/kcal)	645	0.8 (0.01, 1.7)	0.05
Vitamin B_{12} (µg/day/kcal)	645	-0.03 (-0.08, 0.02)	0.2
Folate (µg/day/kcal)	645	0.002 (-0.001, 0.01)	0.1
Protein (µg/day/kcal)	645	0.01 (-0.01, 0.03)	0.3
Alcohol (µg/day/kcal)	645	-0.01 (-0.03, 0.02)	0.5
Fruit (g/day/kcal)	639	0.0001 (-0.001, 0.002)	0.9
Vegetable (g/day/kcal)	640	0.002 (-0.0004, 0.004)	0.1
Fruit and vegetable (g/day/kcal)	639	0.001 (-0.001, 0.002)	0.4

Supplementary Table S3-5 (cont.) Association between LINE-1 methylation and individual characteristics of study subjects in the SBC/EPICURO Study

Variables	N	β (95% CI)*	P-values
Toenail trace elements‡			
Aluminium (µg/g)	658	-0.003 (-0.01, 0.002)	0.2
Arsenic (µg/g)	659	-3.3 (-5.7, -1.0)	0.006
Cadmium (µg/g)	659	0.1 (-0.3, 0.6)	0.7
Chromium (µg/g)	659	-0.01 (-0.05, 0.03)	0.7
Copper (µg/g)	659	-0.01 (-0.06, 0.05)	0.8
Iron (µg/g)	658	0.002 (0.001, 0.004)	0.008
Lead (µg/g)	659	-0.05 (-0.1, 0.03)	0.2
Manganese (µg/g)	659	-0.05 (-0.2, 0.06)	0.4
Nickel (µg/g)	659	0.02 (0.01, 0.03)	0.004
Selenium (µg/g)	659	0.4 (-0.6, 1.3)	0.4
Vanadium (µg/g)	651	-0.8 (-2.9, 1.3)	0.4
Zinc (µg/g)	659	-0.001 (-0.004, 0.001)	0.4
***NAT2* phenotype**			
Rapid/Intermediate	389	Ref	
Slow	498	0.2 (-0.1, 0.5)	0.2
Missing data	5		
***GSTM1* genotype**			
(+/+, +/-)	421	Ref	
(-/-)	462	0.02 (-0.3, 0.3)	0.9
Missing data	9		
***GSTT1* genotype**			
(+/+, +/-)	688	Ref	
(-/-)	198	-0.2 (-0.6, 0.2)	0.4
Missing data	6		

*Adjusted for age, gender, and region.

†Data available for those who completed food frequency questionnaire.

‡Data available for those who provided toe nail for trace element assessment.

Note: the exposure contrast for trace elements is 1-µg/g and for dietary variables is 1-µg/day/kcal.

Supplementary Table S3-6 Association between LINE-1 methylation levels and 15 single nucleotide polymorphisms in genes involved in the one-carbon metabolism pathway with nonsignificant results

Gene	dbSNP Identifier	N	Genotype	Additive MOI β (95% CI)*	P-value	N	Genotype	Codominant MOI β (95% CI)*	Global P-Value†	N	Genotype	Dominant MOI β (95% CI)*	P-value	N	Genotype	Recessive MOI β (95% CI)*	P-value
DNMT3A	rs11683424	872	C>T	-0.3 (-0.6, 0.1)	0.1	677	CC	Ref	0.3	677	CC	Ref		855	CC/CT	Ref	
						178	CT	-0.2 (-0.6, 0.2)		195	CT/TT	-0.2 (-0.6, 0.1)	0.2	17	TT	-0.8 (-2.0, 0.3)	0.2
						17	TT	-0.9 (-2.0, 0.3)									
DNMT3A	rs1550117	872	G>A	0.2 (-0.2, 0.7)	0.3	743	GG	Ref	0.5	743	GG	Ref		868	GG/GA	Ref	
						125	GA	0.3 (-0.2, 0.7)		129	GA/AA	0.3 (-0.2, 0.7)	0.3	4	AA	0.3 (-2.1, 2.6)	0.8
						4	AA	0.3 (-2.0, 2.7)									
ATIC	rs4369857	872	A>C	0.4 (-0.2, 0.9)	0.2	796	AA	Ref	0.3	796	AA	Ref		871	AA/AC	Ref	
						75	AC	0.4 (-0.1, 1.0)		76	AC/CC	0.4 (-0.1, 0.9)	0.1	1	CC	0.1 (-4.6, 4.8)	1.0
						1	CC	0.2 (-4.5, 4.8)									
ATIC	rs7563206	871	C>T	-0.2 (-0.4, 0.01)	0.07	242	CC	Ref	0.2	242	CC	Ref		681	CC/CT	Ref	
						439	CT	-0.2 (-0.5, 0.2)		629	CT/TT	-0.3 (-0.6, 0.1)	0.2	190	TT	-0.3 (-0.7, 0.1)	0.1
						190	TT	-0.4 (-0.9, 0.03)									
ATIC	rs1880586	872	A>G	-0.2 (-0.4, 0.01)	0.06	242	AA	Ref	0.2	242	AA	Ref		681	AA/AG	Ref	
						439	AG	-0.2 (-0.6, 0.2)		630	AG/GG	-0.3 (-0.6, 0.1)	0.1	191	GG	-0.3 (-0.7, 0.1)	0.1
						191	GG	-0.4 (-0.9, 0.02)									
ATIC	rs4673991	872	C>T	0.1 (-0.1, 0.4)	0.3	394	CC	Ref	0.5	394	CC	Ref		789	CC/CT	Ref	
						395	CT	0.09 (-0.2, 0.4)		478	CT/TT	0.1 (-0.2, 0.5)	0.4	83	TT	0.3 (-0.3, 0.8)	0.3
						83	TT	0.3 (-0.3, 0.9)									
ATIC	rs4673993	872	T>C	0.1 (-0.1, 0.4)	0.3	394	TT	Ref	0.5	394	TT	Ref		789	TT/TC	Ref	
						395	TC	0.1 (-0.2, 0.4)		478	TC/CC	0.1 (-0.2, 0.5)	0.4	83	CC	0.3 (-0.3, 0.8)	0.3
						83	CC	0.3 (-0.3, 0.9)									
ATIC	rs4672768	870	G>A	0.1 (-0.1, 0.4)	0.4	393	GG	Ref	0.6	393	GG	Ref		787	GG/GA	Ref	
						394	GA	0.1 (-0.2, 0.4)		477	GA/AA	0.1 (-0.2, 0.4)	0.5	83	AA	0.3 (-0.3, 0.8)	0.3
						83	AA	0.3 (-0.3, 0.9)									
ATIC	rs10498036	872	G>T	0.1 (-0.1, 0.3)	0.3	315	GG	Ref	0.5	315	GG	Ref		727	GG/GT	Ref	
						412	GT	0.2 (-0.1, 0.6)		557	GT/TT	0.2 (-0.1, 0.5)	0.2	145	TT	0.1 (-0.3, 0.5)	0.7
						145	TT	0.2 (-0.3, 0.7)									
ATIC	rs7604984	872	A>G	0.1 (-0.08, 0.4)	0.2	313	AA	Ref	0.3	313	AA	Ref		727	AA/AG	Ref	
						414	AG	0.3 (-0.1, 0.6)		559	AG/GG	0.3 (-0.1, 0.6)	0.1	145	GG	0.1 (-0.3, 0.5)	0.7
						145	GG	0.2 (-0.2, 0.7)									
MTHFS	rs1077965	872	T>C	0.01 (-0.2, 0.2)	0.9	311	TT	Ref	0.2	311	TT	Ref		725	TT/TC	Ref	
						414	TC	0.3 (-0.07, 0.6)		561	TC/CC	0.2 (-0.1, 0.5)	0.3	147	CC	-0.2 (-0.7, 0.2)	0.3
						147	CC	-0.1 (-0.5, 0.4)									
MTHFS	rs11855092	872	G>A	-0.03 (-0.3, 0.2)	0.8	509	GG	Ref	0.2	509	GG	Ref		816	GG/GA	Ref	
						307	GA	0.2 (-0.2, 0.5)		363	GA/AA	0.1 (-0.2, 0.4)	0.7	56	AA	-0.5 (-1.1, 0.2)	0.2
						56	AA	-0.4 (-1.1, 0.3)									
PEMT	rs4646340	872	A>G	0.08 (-0.2, 0.3)	0.5	341	AA	Ref	0.1	341	AA	Ref		757	AA/AG	Ref	
						416	AG	0.3 (-0.03, 0.7)		531	AG/GG	0.2 (-0.1, 0.6)	0.2	115	GG	-0.2 (-0.7, 0.3)	0.4
						115	GG	-0.02 (-0.5, 0.5)									
PEMT	rs7215833	872	C>T	0.09 (-0.1, 0.3)	0.5	351	CC	Ref	0.1	351	CC	Ref		763	CC/CT	Ref	
						412	CT	0.3 (-0.01, 0.7)		521	CT/TT	0.3 (-0.1, 0.6)	0.1	109	TT	-0.2 (-0.7, 0.3)	0.5
						109	TT	-0.01 (-0.5, 0.5)									
SLC19A1	rs2838965	870	G>A	0.1 (-0.3, 0.4)	0.4	296	GG	Ref	0.1	296	GG	Ref		708	GG/GA	Ref	
						412	GA	0.4 (0.04, 0.8)		574	GA/AA	0.3(-0.04, 0.6)	0.1	162	AA	-0.1 (-0.5, 0.3)	0.5
						162	AA	0.1 (-0.4, 0.5)									

MAF minor allele frequency; MOI mode of inheritance.

*Adjusted for age, gender, region and smoking status

†Global p-value was estimated by using a two-degrees of freedom likelihood-ratio test.

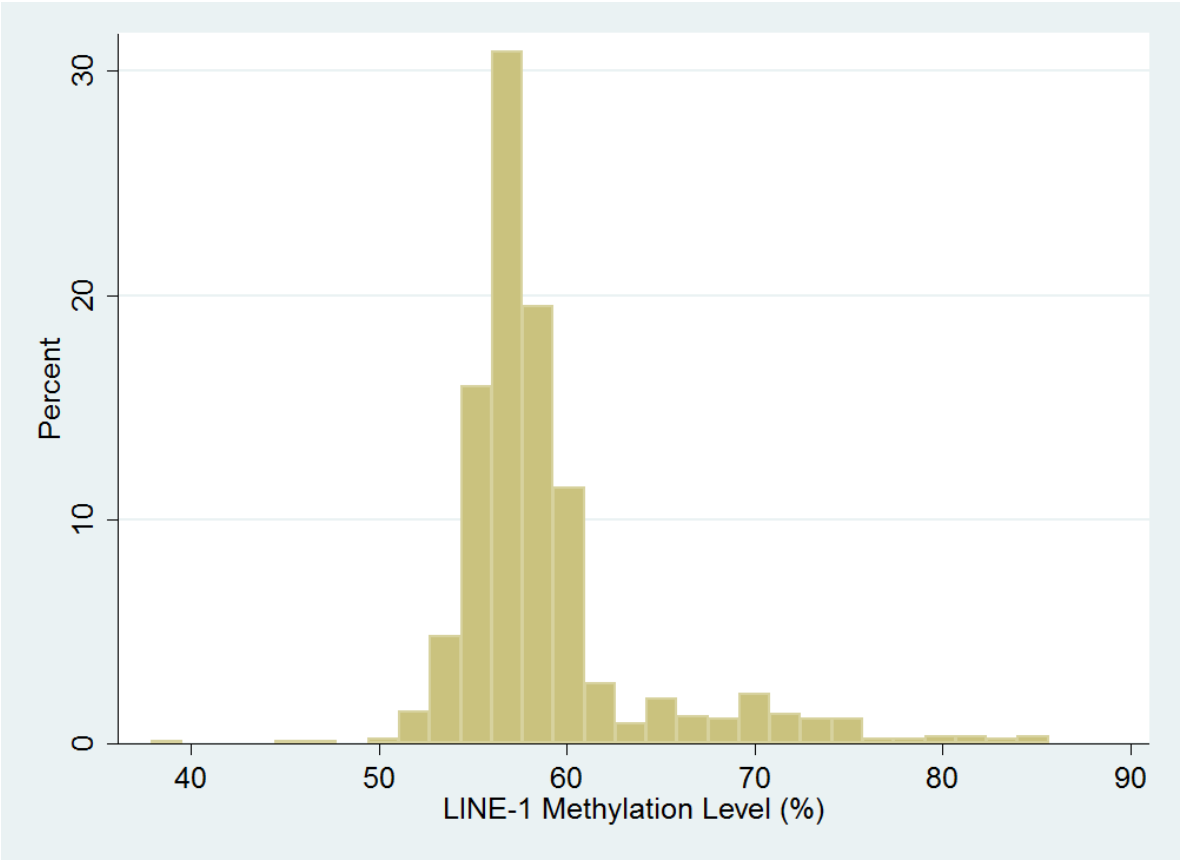
Supplementary Table S3-7 Multivariable model of association between LINE-1 methylation and individual characteristics, toenail trace elements and single nucleotide polymorphisms among study subjects in the SBC/EPICURO Study

Variable	N	β (95% CI)*	P-value	Global p-value†
Age (years)	601	-0.01 (-0.03, 0.01)	0.2	
Gender				
Male	534	Ref		
Female	67	-1.0 (-1.7, -0.3)	0.006	
Region				
Barcelona	131	Ref		
Valles	90	-0.05 (-0.7, 0.6)	0.9	
Elche	57	-0.4 (-1.2, 0.3)	0.3	
Tenerife	82	0.2 (-0.5, 0.9)	0.5	
Asturias	241	0.1 (-0.4, 0.7)	0.6	
Tobacco Type				
Non-smoker	187	Ref		
Blond only	76	-0.5 (-1.2, 0.2)	0.1	
Black only	156	0.09 (-0.5, 0.7)	0.7	
Blond and black	118	-0.6 (-1.2, 0.05)	0.07	
Unknown	64	-0.07 (-0.8, 0.7)	0.9	
Toenail Trace Element				
Arsenic (µg/g)	601	-4.8 (-7.8, -1.8)	0.002	
Iron (µg/g)	601	0.003 (0.001, 0.01)	0.0004	
Nickel (µg/g)	601	0.01 (0.004, 0.03)	0.005	
Gene, dbSNP identifier				
***DNMT3A*-rs7581217,** C>T	601	0.3 (0.003, 0.6)	0.048	
***AS3MT*-rs7085104**				
AA/AG	518	Ref		
GG	83	0.5 (-0.07, 1.1)	0.09	
***MTHFS*-rs1380642**				
CC	409	Ref		
CT	167	0.4 (-0.02, 0.9)	0.06	0.005
TT	25	-1.2 (-2.2, -0.2)	0.02	
***SLC19A1*-rs914238**				
TT	159	Ref		
TC	293	0.3 (-0.1, 0.8)	0.2	0.07
CC	149	-0.2 (-0.8, 0.3)	0.5	
***TCN2*-rs9621049**				
CC/CT	593	Ref		
TT	8	4.3 (0.9, 7.6)	0.01	

Supplementary Table S3-7 (cont.) Multivariable model of association between LINE-1 methylation and individual characteristics, toenail trace elements and single nucleotide polymorphisms among study subjects in the SBC/EPICURO Study

Variable	N	β (95% CI)*	P-value	Global p-value†
***TCN2*-rs9606756**				
AA/AG	594	Ref		
GG	7	-2.3 (-7.1, 2.5)	0.3	
***TCN2*-rs4820887**				
GG/GA	595	Ref		
AA	6	-0.6 (-6.7, 5.6)	0.9	

*Fully adjusted robust regression coefficient.
†Global p-value was estimated by using a two-degrees of freedom likelihood-ratio test.



Supplementary Figure S3-1 Distribution of LINE-1 methylation among study subjects in the SBC/EPICURO study

SUPPLEMENTARY MATERIALS CHAPTER IV

For Supplementary Table S4-1, please see Supplementary Table S3-1

Supplementary Table S4-2 Association between individual LINE-1 CpG position and urothelial carcinoma of the bladder risk in the SBC/EPICURO study

Methylation	Unadjusted Model						Adjusted Model*					
in tertiles†	Cases	Controls	OR	95% CI		P-value	Tertiles	Cases	Controls	OR	95% CI	P-value
LINE-1 CpG1												
T1	344	298	1.20	0.96	1.50	0.1	T1	344	297	1.15	0.91 1.46	0.2
T2	286	297	1 Referent				T2	284	296	1 Referent		
T3	322	297	1.13	0.90	1.41	0.3	T3	318	296	1.10	0.87 1.40	0.4
LINE-1 CpG2												
T1	337	298	1.32	1.05	1.66	0.02	T1	334	297	1.24	0.98 1.58	0.08
T2	255	297	1 Referent				T2	255	296	1 Referent		
T3	360	297	1.41	1.12	1.77	0.003	T3	357	296	1.42	1.11 1.80	0.005
LINE-1 CpG3												
T1	329	298	1.18	0.94	1.48	0.1	T1	326	296	1.13	0.89 1.44	0.3
T2	277	297	1 Referent				T2	277	296	1 Referent		
T3	346	297	1.25	1.00	1.57	0.05	T3	343	297	1.20	0.94 1.52	0.1
LINE-1 CpG4												
T1	317	298	1.15	0.92	1.45	0.2	T1	315	296	1.15	0.91 1.47	0.2
T2	274	297	1 Referent				T2	272	297	1 Referent		
T3	361	297	1.32	1.05	1.65	0.02	T3	359	296	1.33	1.05 1.68	0.02

*Model adjusted for age, gender, region, smoking status (never, occasional, former and current smoker).

†Tertile cut-offs: CpG1: 76.78%, 78.23%; CpG2: 45.59%, 48.07%; CpG3: 58.25%, 60.18%; CpG4: 45.44%, 48.38%.

Supplementary Table S4-3 Association between LINE-1 methylation and risk of bladder cancer stratified by tumor subphenotypes in the SBC/EPICURO study

LINE-1 methylation (tertiles)	Cases	Controls	OR*	95% CI		*P*
Low-grade NNIBC						
T1 (<56.68)	190	296	1.33	1.00	1.77	0.05
T2 (56.68-<58.65)	136	297	1 Referent			
T3 (≥58.65)	194	296	1.37	1.03	1.82	0.03
High-grade NMIBC						
T1 (<56.68)	63	296	1.12	0.74	1.69	0.58
T2 (56.68-<58.65)	54	297	1 Referent			
T3 (≥58.65)	59	296	1.14	0.75	1.73	0.53
MIBC						
T1 (<56.68%)	66	296	1.16	0.77	1.74	0.50
T2 (56.68-<58.65%)	55	297	1 Referent			
T3 (≥58.65%)	83	296	1.62	1.09	2.41	0.02

*Adjusted model for age, gender, region and smoking status (never, occasional, former and current smoker).

P value for heterogeneity: low- vs high-grade NMIBC = 0.5; low-grade NMIBC vs MIBC = 0.6; high-grade NMIBC vs MIBC=0.4.

NMIBC, non-muscle-invasive bladder cancer; MIBC, muscle-invasive bladder cancer.

Supplementary Table S4-4 Association between LINE-1 methylation and urothelial carcinoma of the bladder risk stratified by age, gender and smoking status in the SBC/EPICURO study

LINE-1 methylation (tertiles)	Cases	Controls	OR*	95% CI		P-value	*P* interaction
Age							
Age <60 years							
T1 (<56.68%)	84	91	1.12	0.69	1.82	0.6	0.2
T2 (56.68-<58.65%)	53	72	1 Referent				
T3 (≥58.65%)	72	90	0.93	0.56	1.53	0.7	
Age 60 - 69 years							
T1 (<56.68%)	124	107	1.25	0.84	1.86	0.3	
T2 (56.68-<58.65%)	108	119	1 Referent				
T3 (≥58.65%)	109	114	1.11	0.74	1.66	0.6	
Age ≥ 70 years							
T1 (<56.68%)	127	98	1.41	0.95	2.09	0.08	
T2 (56.68-<58.65%)	99	106	1 Referent				
T3 (≥58.65%)	170	92	1.96	1.33	2.88	0.0006	
Gender							
LINE-1 methylation (tertiles)	Cases	Controls	OR†	95% CI		P-value	*P* interaction
Male							
T1 (<56.68%)	285	257	1.26	0.98	1.64	0.08	0.5
T2 (56.68-<58.65%)	230	265	1 Referent				
T3 (≥58.65%)	305	267	1.28	0.99	1.66	0.06	
Female							
T1 (<56.68%)	50	39	1.28	0.65	2.50	0.5	
T2 (56.68-<58.65%)	30	32	1 Referent				
T3 (≥58.65%)	46	29	1.76	0.87	3.56	0.1	
Smoking status							
LINE-1 methylation (tertiles)	Cases	Controls	OR‡	95% CI		P-value	*P* interaction
Never smoker							
T1 (<56.68%)	54	84	2.06	1.17	3.62	0.01	0.2
T2 (56.68-<58.65%)	30	94	1 Referent				
T3 (≥58.65%)	53	77	2.05	1.16	3.63	0.01	
Ever smoker							
T1 (<56.68%)	281	212	1.18	0.90	1.53	0.2	
T2 (56.68-<58.65%)	230	203	1 Referent				
T3 (≥58.65%)	298	219	1.21	0.93	1.57	0.2	

*Adjusted for gender, region and smoking status (never, occasional, former and current smoker). Age categorized by the median age was used to calculate the interaction p-value.
†Adjusted for age, region and smoking status (never, occasional, former and current smoker).
‡Adjusted for age, gender and region. Smoking status categorized as never smoker and ever smoker was used to calculate the interaction p-value.

Supplementary Table S4-5 Association between LINE-1 methylation and urothelial carcinoma of the bladder risk adjusted for tobacco type, B vitamins, protein, alcohol intakes, trace elements, and variations in *GSTM1* and *GSTT1* in the SBC/EPICURO study

Adjusted for:	LINE-1 methylation (tertiles)	Cases	Controls	OR	95% CI		P-value	*P* interaction
Tobacco type*	T1 (<56.68%)	335	296	1.28	1.00	1.63	0.05	0.2
	T2 (56.68-<58.65%)	260	297	1 Referent				
	T3 (≥58.65%)	351	296	1.39	1.09	1.78	0.008	
Vitamin B_1 intake (µg/day/kcal)†	T1 (<56.68%)	249	220	1.12	0.84	1.48	0.4	0.5
	T2 (56.68-<58.65%)	194	204	1 Referent				
	T3 (≥58.65%)	268	220	1.23	0.93	1.62	0.2	
Vitamin B_2 intake (µg/day/kcal)†	T1 (<56.68%)	249	220	1.12	0.84	1.48	0.4	0.7
	T2 (56.68-<58.65%)	194	204	1 Referent				
	T3 (≥58.65%)	268	220	1.23	0.93	1.63	0.1	
Vitamin B_3 intake (µg/day/kcal)†	T1 (<56.68%)	249	220	1.12	0.85	1.49	0.4	0.5
	T2 (56.68-<58.65%)	194	204	1 Referent				
	T3 (≥58.65%)	268	220	1.24	0.94	1.64	0.1	
Vitamin B_6 intake (µg/day/kcal)†	T1 (<56.68%)	249	220	1.11	0.83	1.47	0.5	0.7
	T2 (56.68-<58.65%)	194	204	1 Referent				
	T3 (≥58.65%)	268	220	1.24	0.93	1.63	0.1	
Vitamin B_{12} intake (µg/day/kcal)†	T1 (<56.68%)	249	220	1.12	0.84	1.49	0.4	0.8
	T2 (56.68-<58.65%)	194	204	1 Referent				
	T3 (≥58.65%)	268	220	1.24	0.94	1.65	0.1	
Folate intake (µg/day/kcal)†	T1 (<56.68%)	249	220	1.11	0.84	1.48	0.5	0.4
	T2 (56.68-<58.65%)	194	204	1 Referent				
	T3 (≥58.65%)	268	220	1.24	0.94	1.64	0.1	
Protein intake (µg/day/kcal)†	T1 (<56.68%)	249	220	1.13	0.85	1.50	0.4	0.7
	T2 (56.68-<58.65%)	194	204	1 Referent				
	T3 (≥58.65%)	268	220	1.25	0.95	1.66	0.1	

Supplementary Table S4-5 (cont.) Association between LINE-1 methylation and urothelial carcinoma of the bladder risk adjusted for tobacco type, B vitamins, protein, alcohol intakes, trace elements, and variations in GSTM1 and GSTT1 in the SBC/EPICURO study

Adjusted for:	LINE-1 methylation (tertiles)	Cases	Controls	OR	95% CI		P-value	*P* interaction
Alcohol intake (µg/day/kcal)†	T1 (<56.68%)	249	220	1.11	0.84	1.48	0.5	0.9
	T2 (56.68-<58.65%)	194	204	1 Referent				
	T3 (≥58.65%)	268	220	1.22	0.92	1.61	0.2	
Aluminum†	T1 (<56.68%)	257	220	1.17	0.89	1.55	0.2	0.5
	T2 (56.68-<58.65%)	207	208	1 Referent				
	T3 (≥58.65%)	277	230	1.20	0.91	1.58	0.2	
Arsenic†	T1 (<56.68%)	257	221	1.14	0.87	1.51	0.3	0.9
	T2 (56.68-<58.65%)	207	208	1 Referent				
	T3 (≥58.65%)	277	230	1.17	0.89	1.54	0.3	
Cadmium†	T1 (<56.68%)	257	221	1.17	0.88	1.54	0.3	0.4
	T2 (56.68-<58.65%)	207	208	1 Referent				
	T3 (≥58.65%)	277	230	1.21	0.92	1.59	0.2	
Chromium†	T1 (<56.68%)	257	221	1.13	0.86	1.50	0.4	0.5
	T2 (56.68-<58.65%)	207	208	1 Referent				
	T3 (≥58.65%)	277	230	1.17	0.89	1.54	0.3	
Copper†	T1 (<56.68%)	257	221	1.15	0.87	1.53	0.3	0.9
	T2 (56.68-<58.65%)	207	208	1 Referent				
	T3 (≥58.65%)	277	230	1.18	0.90	1.55	0.2	
Iron†	T1 (<56.68%)	255	221	1.15	0.87	1.52	0.3	0.4
	T2 (56.68-<58.65%)	206	207	1 Referent				
	T3 (≥58.65%)	276	230	1.19	0.90	1.57	0.2	
Lead†	T1 (<56.68%)	257	221	1.13	0.86	1.50	0.4	0.5
	T2 (56.68-<58.65%)	207	208	1 Referent				
	T3 (≥58.65%)	277	230	1.17	0.89	1.54	0.3	

Supplementary Table S4-5 (cont.) Association between LINE-1 methylation and urothelial carcinoma of the bladder risk adjusted for tobacco type, B vitamins, protein, alcohol intakes, trace elements, and variations in GSTM1 and GSTT1 in the SBC/EPICURO study

Adjusted for:	LINE-1 methylation (tertiles)	Cases	Controls	OR	95% CI		P-value	*P* interaction
Manganese†	T1 (<56.68%)	257	221	1.16	0.88	1.53	0.3	0.2
	T2 (56.68-<58.65%)	207	208	1 Referent				
	T3 (≥58.65%)	277	230	1.20	0.91	1.58	0.2	
Nickel†	T1 (<56.68%)	257	221	1.13	0.86	1.50	0.4	0.9
	T2 (56.68-<58.65%)	207	208	1 Referent				
	T3 (≥58.65%)	277	230	1.16	0.88	1.53	0.3	
Selenium†	T1 (<56.68%)	257	221	1.14	0.86	1.50	0.4	0.5
	T2 (56.68-<58.65%)	207	208	1 Referent				
	T3 (≥58.65%)	277	230	1.17	0.89	1.54	0.3	
Vanadium†	T1 (<56.68%)	248	218	1.12	0.85	1.49	0.4	0.3
	T2 (56.68-<58.65%)	206	205	1 Referent				
	T3 (≥58.65%)	270	228	1.16	0.88	1.53	0.3	
Zinc†	T1 (<56.68%)	257	221	1.15	0.87	1.52	0.3	0.9
	T2 (56.68-<58.65%)	207	208	1 Referent				
	T3 (≥58.65%)	277	230	1.18	0.89	1.55	0.2	
GSTM1†	T1 (<56.68%)	332	296	1.24	0.98	1.58	0.08	0.5
	T2 (56.68-<58.65%)	258	291	1 Referent				
	T3 (≥58.65%)	346	293	1.33	1.04	1.69	0.02	
GSTT1†	T1 (<56.68%)	335	295	1.26	0.99	1.60	0.06	0.5
	T2 (56.68-<58.65%)	259	295	1 Referent				
	T3 (≥58.65%)	348	293	1.34	1.05	1.70	0.02	

*Model adjusted for age, gender, region, tobacco type.
†Variable included in a model adjusted for age, gender, region, smoking status (never, occasional, former and current smoker).

Supplementary Table S4-6 Corrected likelihood ratio test p-values of the interaction between LINE-1 methylation and SNPs in one-carbon metabolism pathway and bladder cancer susceptibility genes and in the SBS/EPICURO study

SNPs modeled in additive mode of inheritance							
dbSNP (N=524)	Chromosome	Gene	N	MAF	*P* HWE	*P* LRT	Corrected *P* LRT
rs2124344	17	*PEMT*	1797	0.36	0.77	0.0003	0.2
rs4646350	17	*PEMT*	1798	0.36	0.83	0.0007	0.3
rs10179873	2	*ATIC*	1798	0.30	0.63	0.002	0.6
rs11892429	2	*ATIC*	1798	0.29	1.00	0.002	0.6
rs10197559	2	*ATIC*	1788	0.29	0.87	0.002	0.7
rs1983462	2	*ATIC*	1798	0.31	0.47	0.002	0.7
rs4646341	17	*PEMT*	1795	0.37	0.56	0.002	0.7
rs10197653	2	*ATIC*	1798	0.29	0.74	0.003	0.8
rs6706415	2	*ATIC*	1798	0.31	0.81	0.003	0.8
rs4646340	17	*PEMT*	1798	0.37	0.51	0.003	0.8
rs7215833	17	*PEMT*	1798	0.36	0.51	0.005	0.9
rs7581217	2	*DNMT3A*	1798	0.39	0.62	0.006	0.9
rs11683424	2	*DNMT3A*	1798	0.12	0.20	0.007	1.0
rs4646359	17	*PEMT*	1798	0.46	0.15	0.010	1.0
rs7604984	2	*ATIC*	1798	0.40	0.62	0.010	1.0
rs798766	4	*TMEM129-TACC3-FGFR3*	1798	0.18	0.21	0.01	1.0
rs11656215	17	*PEMT*	1798	0.46	0.20	0.01	1.0
rs10498036	2	*ATIC*	1798	0.40	0.57	0.01	1.0
rs11855092	15	*MTHFS*	1798	0.24	0.31	0.01	1.0
rs4673993	2	*ATIC*	1798	0.32	0.31	0.02	1.0
rs1077965	15	*MTHFS*	1797	0.41	0.58	0.02	1.0
rs4244599	17	*PEMT*	1774	0.47	0.34	0.02	1.0
rs1880580	15	*MTHFS*	1798	0.31	0.38	0.02	1.0
rs435689	15	*MTHFS*	1798	0.49	0.50	0.02	1.0
rs4924922	17	*PEMT*	1798	0.37	0.43	0.02	1.0
rs4673991	2	*ATIC*	1797	0.32	0.31	0.02	1.0
rs282814	15	*MTHFS*	1798	0.22	0.84	0.02	1.0
rs4672768	2	*ATIC*	1794	0.32	0.31	0.02	1.0
rs7560488	2	*DNMT3A*	1731	0.48	0.15	0.02	1.0
rs12997662	2	*ATIC*	1798	0.34	0.26	0.02	1.0
rs7563206	2	*ATIC*	1796	0.47	0.84	0.03	1.0
rs4646344	17	*PEMT*	1798	0.46	0.45	0.03	1.0
rs4817580	21	*GART*	1797	0.10	0.70	0.03	1.0
rs6057645	20	*DNMT3B*	1797	0.04	0.22	0.04	1.0
rs9890064	17	*PEMT*	1798	0.43	0.41	0.04	1.0
rs12905663	15	*MTHFS*	1788	0.29	0.80	0.04	1.0
rs4646385	17	*PEMT*	1798	0.45	0.37	0.04	1.0
rs11869600	17	*PEMT*	1796	0.37	0.38	0.04	1.0
rs1880586	2	*ATIC*	1797	0.47	0.89	0.05	1.0
rs6495441	15	*MTHFS*	1798	0.25	0.93	0.05	1.0

Supplementary Table S4-6 (cont.) Corrected likelihood ratio test p-values of the interaction between LINE-1 methylation and SNPs in one-carbon metabolism pathway and bladder cancer susceptibility genes and in the SBS/EPICURO study

dbSNP (N=524)	Chromosome	Gene	N	MAF	*P* HWE	*P* LRT	Corrected *P* LRT
rs3760188	17	*PEMT*	1798	0.46	0.38	0.05	1.0
rs11871738	17	*PEMT*	1798	0.38	0.67	0.06	1.0
rs6749992	2	*DNMT3A*	1798	0.47	0.79	0.06	1.0
rs4646404	17	*PEMT*	1792	0.35	0.26	0.06	1.0
rs2289209	3	*CHDH*	1798	0.04	0.67	0.06	1.0
rs740234	22	*TCN2*	1798	0.23	0.39	0.07	1.0
rs9835128	3	*CHDH*	1797	0.16	0.38	0.08	1.0
rs750546	17	*PEMT*	1773	0.45	0.19	0.08	1.0
rs1814175	11	*FOLH1*	1793	0.40	0.40	0.08	1.0
rs7605753	2	*DNMT3A*	1797	0.47	0.68	0.08	1.0
rs10380	5	*MTRR*	1793	0.10	0.56	0.08	1.0
rs17824591	14	*MTHFD1*	1796	0.23	0.63	0.08	1.0
rs10839295	11	*FOLH1*	1797	0.40	0.48	0.08	1.0
rs3862350	11	*FOLH1*	1765	0.40	0.15	0.09	1.0
rs2733106	15	*MTHFS*	1793	0.15	0.69	0.09	1.0
rs6119285	20	*DNMT3B*	1795	0.04	0.20	0.1	1.0
rs748196	17	*PEMT*	1795	0.44	0.89	0.1	1.0
rs1917311	11	*FOLH1*	1753	0.40	0.72	0.1	1.0
rs7111215	11	*FOLH1*	1774	0.40	0.52	0.1	1.0
rs2183601	21	*SLC19A1*	1797	0.20	0.92	0.1	1.0
rs282776	15	*MTHFS*	1798	0.36	0.47	0.1	1.0
rs282787	15	*MTHFS*	1798	0.04	0.34	0.1	1.0
rs2586153	15	*MTHFS*	1777	0.15	0.59	0.1	1.0
rs9606756	22	*TCN2*	1798	0.12	0.87	0.1	1.0
rs16988828	22	*TCN2*	1798	0.09	0.09	0.1	1.0
rs2838973	21	*SLC19A1*	1798	0.20	0.92	0.1	1.0
rs7583409	2	*DNMT3A*	1795	0.36	0.38	0.1	1.0
rs4531931	2	*ATIC*	1796	0.31	0.23	0.1	1.0
rs2150460	21	*SLC19A1*	1798	0.20	1.00	0.1	1.0
rs10509760	10	*AS3MT*	1798	0.13	0.88	0.1	1.0
rs2424913	20	*DNMT3B*	1797	0.37	0.39	0.1	1.0
rs4476347	2	*ATIC*	1798	0.25	0.28	0.1	1.0
rs3740394	10	*AS3MT*	1797	0.13	1.00	0.1	1.0
rs1380642	15	*MTHFS*	1798	0.18	0.57	0.1	1.0
rs4434082	21	*SLC19A1*	1797	0.20	1.00	0.1	1.0
rs7102702	11	*FOLH1*	1741	0.04	1.00	0.2	1.0
rs1950902	14	*MTHFD1*	1798	0.15	1.00	0.2	1.0
rs4779148	15	*MTHFS*	1798	0.10	0.45	0.2	1.0
rs9976878	21	*SLC19A1*	1797	0.20	1.00	0.2	1.0
rs11191439	10	*AS3MT*	1795	0.12	0.75	0.2	1.0

Supplementary Table S4-6 (cont.) Corrected likelihood ratio test p-values of the interaction between LINE-1 methylation and SNPs in one-carbon metabolism pathway and bladder cancer susceptibility genes and in the SBS/EPICURO study

dbSNP (N=524)	Chromosome	Gene	N	MAF	*P* HWE	*P* LRT	Corrected *P* LRT
rs9306139	21	*SLC19A1*	1796	0.20	1.00	0.2	1.0
rs1802059	5	*MTRR*	1792	0.36	0.60	0.2	1.0
rs1014971	22	*CBX6 APOBEC3A*	1797	0.30	0.23	0.2	1.0
rs3862342	11	*FOLH1*	1795	0.28	0.93	0.2	1.0
rs5749135	22	*TCN2*	1798	0.43	0.07	0.2	1.0
rs10932605	2	*ATIC*	1798	0.14	0.39	0.2	1.0
rs4369857	2	*ATIC*	1798	0.04	1.00	0.2	1.0
rs897453	17	*PEMT*	1790	0.47	0.54	0.2	1.0
rs17209637	15	*MTHFS*	1794	0.26	0.79	0.2	1.0
rs6058897	20	*DNMT3B*	1798	0.44	0.17	0.2	1.0
rs443394	15	*MTHFS*	1798	0.42	0.30	0.2	1.0
rs1604503	15	*MTHFS*	1798	0.15	0.59	0.2	1.0
rs12995968	2	*DNMT3A*	1798	0.04	0.66	0.2	1.0
rs6757920	2	*ATIC*	1794	0.48	0.09	0.2	1.0
rs4925048	17	*PEMT*	1797	0.10	0.17	0.2	1.0
rs6760069	2	*ATIC*	1797	0.15	0.35	0.2	1.0
rs2866358	11	*FOLH1*	1781	0.38	0.35	0.2	1.0
rs582172	15	*MTHFS*	1798	0.42	0.45	0.2	1.0
rs10948059	6	*GNMT*	1778	0.49	0.54	0.2	1.0
rs11040416	11	*FOLH1*	1798	0.42	0.58	0.2	1.0
rs2424906	20	*DNMT3B*	1798	0.37	0.28	0.2	1.0
rs2301955	22	*TCN2*	1794	0.43	0.10	0.2	1.0
rs9980967	21	*SLC19A1*	1797	0.11	0.38	0.2	1.0
rs2294008	8	*PSCA*	1798	0.46	0.73	0.2	1.0
rs1127717	3	*ALDH1L1*	1793	0.24	1.00	0.2	1.0
rs1164685	11	*FOLH1*	1793	0.38	0.47	0.2	1.0
rs12898670	15	*MTHFS*	1796	0.34	0.70	0.2	1.0
rs9462856	6	*GNMT*	1798	0.42	0.53	0.2	1.0
rs17211644	15	*MTHFS*	1798	0.10	0.72	0.2	1.0
rs1074390	15	*MTHFS*	1798	0.38	0.77	0.2	1.0
rs2838964	21	*SLC19A1*	1798	0.06	0.77	0.2	1.0
rs2733103	15	*MTHFS*	1797	0.15	0.51	0.2	1.0
rs4911108	20	*DNMT3B*	1793	0.28	0.87	0.2	1.0
rs6518253	21	*SLC19A1*	1797	0.46	0.63	0.2	1.0
rs7276295	21	*SLC19A1*	1798	0.06	0.56	0.2	1.0
rs1495741	8	*NAT2*	1798	0.24	1.00	0.2	1.0
rs9621049	22	*TCN2*	1798	0.11	0.86	0.2	1.0
rs6058896	20	*DNMT3B*	1797	0.09	0.67	0.2	1.0
rs588458	11	*FOLH1*	1772	0.38	0.52	0.2	1.0
rs34048824	2	*DNMT3A*	1797	0.51	0.74	0.2	1.0

Supplementary Table S4-6 (cont.) Corrected likelihood ratio test p-values of the interaction between LINE-1 methylation and SNPs in one-carbon metabolism pathway and bladder cancer susceptibility genes and in the SBS/EPICURO study

dbSNP (N=524)	Chromosome	Gene	N	MAF	*P* HWE	*P* LRT	Corrected *P* LRT
rs914238	21	*SLC19A1*	1798	0.49	0.89	0.3	1.0
rs11040421	11	*FOLH1*	1798	0.14	0.78	0.3	1.0
rs2115536	15	*MTHFS*	1798	0.49	0.22	0.3	1.0
rs4144700	11	*FOLH1*	1798	0.38	0.47	0.3	1.0
rs910085	20	*DNMT3B*	1797	0.29	0.93	0.3	1.0
rs166868	15	*MTHFS*	1796	0.37	0.94	0.3	1.0
rs6058894	20	*DNMT3B*	1797	0.08	1.00	0.3	1.0
rs1055345	21	*SLC19A1*	1797	0.29	0.10	0.3	1.0
rs7586294	2	*DNMT3A*	1797	0.47	0.73	0.3	1.0
rs16971253	15	*MTHFS*	1797	0.10	1.00	0.3	1.0
rs13427202	2	*DNMT3A*	1797	0.47	0.68	0.3	1.0
rs1256112	14	*MTHFD1*	1798	0.40	0.29	0.3	1.0
rs2838965	21	*SLC19A1*	1792	0.42	0.33	0.3	1.0
rs12797853	11	*FOLH1*	1794	0.13	0.46	0.3	1.0
rs4911107	20	*DNMT3B*	1798	0.31	1.00	0.3	1.0
rs2283873	22	*TCN2*	1779	0.03	0.49	0.3	1.0
rs2305230	3	*ALDH1L1*	1792	0.20	0.30	0.3	1.0
rs10418	22	*TCN2*	1772	0.21	0.41	0.3	1.0
rs3785499	17	*PEMT*	1798	0.48	0.54	0.3	1.0
rs7219568	17	*PEMT*	1798	0.06	0.52	0.3	1.0
rs12797843	11	*FOLH1*	1798	0.13	0.37	0.3	1.0
rs2267163	22	*TCN2*	1794	0.43	0.06	0.3	1.0
rs6058883	20	*DNMT3B*	1797	0.39	0.36	0.3	1.0
rs11887120	2	*DNMT3A*	1798	0.41	0.44	0.3	1.0
rs2424928	20	*DNMT3B*	1798	0.39	0.26	0.3	1.0
rs2115540	15	*MTHFS*	1797	0.49	0.20	0.3	1.0
rs9332	5	*MTRR*	1793	0.12	0.34	0.3	1.0
rs6713377	2	*DNMT3A*	1797	0.47	0.89	0.3	1.0
rs4673965	2	*ATIC*	1798	0.40	0.94	0.3	1.0
rs2424914	20	*DNMT3B*	1798	0.39	0.39	0.3	1.0
rs7124266	11	*FOLH1*	1798	0.30	0.94	0.3	1.0
rs7575625	2	*DNMT3A*	1797	0.47	0.89	0.3	1.0
rs4646364	17	*PEMT*	1797	0.03	0.15	0.3	1.0
rs10839296	11	*FOLH1*	1777	0.25	0.65	0.3	1.0
rs6058891	20	*DNMT3B*	1796	0.39	0.32	0.3	1.0
rs17285431	15	*MTHFS*	1798	0.17	0.08	0.3	1.0
rs7111711	11	*FOLH1*	1798	0.25	0.86	0.3	1.0
rs4532960	10	*AS3MT*	1797	0.44	0.89	0.3	1.0
rs709046	20	*DNMT3B*	1798	0.03	1.00	0.3	1.0
rs10748835	10	*AS3MT*	1798	0.44	0.89	0.3	1.0

Supplementary Table S4-6 (cont.) Corrected likelihood ratio test p-values of the interaction between LINE-1 methylation and SNPs in one-carbon metabolism pathway and bladder cancer susceptibility genes and in the SBS/EPICURO study

dbSNP (N=524)	Chromosome	Gene	N	MAF	*P* HWE	*P* LRT	Corrected *P* LRT
rs4495895	11	*FOLH1*	1751	0.09	0.39	0.3	1.0
NA	1	*MTR_01_ORDER*	1793	0.16	0.81	0.4	1.0
rs10501325	11	*FOLH1*	1798	0.06	0.08	0.4	1.0
rs12121543	1	*MTHFR*	1793	0.22	0.32	0.4	1.0
rs1460177	15	*MTHFS*	1797	0.08	0.37	0.4	1.0
rs11040387	11	*FOLH1*	1785	0.07	0.31	0.4	1.0
rs1801198	22	*TCN2*	1797	0.43	0.08	0.4	1.0
rs473334	1	*CTH*	1793	0.31	0.81	0.4	1.0
rs663649	1	*CTH*	1793	0.31	0.81	0.4	1.0
rs710521	3	*TP63*	1798	0.26	0.93	0.4	1.0
rs7107178	11	*FOLH1*	1796	0.25	0.86	0.4	1.0
rs8101626	19	*DNMT1*	1798	0.39	0.89	0.4	1.0
rs2162560	19	*DNMT1*	1797	0.38	0.94	0.4	1.0
rs2288349	19	*DNMT1*	1797	0.38	0.78	0.4	1.0
rs2035027	15	*MTHFS*	1798	0.16	0.80	0.4	1.0
rs12482346	21	*SLC19A1*	1797	0.48	0.10	0.4	1.0
rs11892646	2	*DNMT3A*	1797	0.11	0.87	0.4	1.0
rs2424921	20	*DNMT3B*	1798	0.39	0.32	0.4	1.0
rs2424922	20	*DNMT3B*	1796	0.39	0.29	0.4	1.0
rs16971450	15	*MTHFS*	1797	0.16	0.80	0.4	1.0
rs6511677	19	*DNMT1*	1797	0.38	0.89	0.4	1.0
rs7117247	11	*FOLH1*	1798	0.06	0.08	0.4	1.0
rs4820887	22	*TCN2*	1798	0.10	0.85	0.4	1.0
rs9789571	2	*ATIC*	1798	0.42	0.89	0.4	1.0
rs6750194	2	*ATIC*	1784	0.07	0.62	0.4	1.0
rs16906158	11	*FOLH1*	1796	0.08	1.00	0.4	1.0
rs10400277	11	*FOLH1*	1774	0.13	0.37	0.4	1.0
rs2275566	1	*MTR*	1793	0.41	0.53	0.4	1.0
rs2116940	19	*DNMT1*	1797	0.08	0.64	0.4	1.0
rs7175620	15	*MTHFS*	1797	0.22	0.14	0.4	1.0
rs1081235	15	*MTHFS*	1798	0.20	0.67	0.4	1.0
rs17279286	15	*MTHFS*	1797	0.05	0.73	0.4	1.0
rs4804122	19	*DNMT1*	1798	0.39	0.52	0.4	1.0
rs515064	1	*CTH*	1793	0.35	0.50	0.4	1.0
rs4779165	15	*MTHFS*	1797	0.16	0.80	0.4	1.0
rs4673981	2	*ATIC*	1798	0.40	1.00	0.4	1.0
rs10163099	15	*MTHFS*	1792	0.26	0.48	0.4	1.0
rs1369703	2	*DNMT3A*	1798	0.44	0.10	0.4	1.0
rs13002567	2	*DNMT3A*	1798	0.28	0.40	0.4	1.0
rs2304429	2	*DNMT3A*	1798	0.43	0.10	0.4	1.0

Supplementary Table S4-6 (cont.) Corrected likelihood ratio test p-values of the interaction between LINE-1 methylation and SNPs in one-carbon metabolism pathway and bladder cancer susceptibility genes and in the SBS/EPICURO study

dbSNP (N=524)	Chromosome	Gene	N	MAF	*P* HWE	*P* LRT	Corrected *P* LRT
rs1805087	1	*MTR*	1793	0.16	0.81	0.4	1.0
rs3788190	21	*SLC19A1*	1795	0.47	0.10	0.4	1.0
rs6087990	20	*DNMT3B*	1797	0.32	0.64	0.4	1.0
rs1550117	2	*DNMT3A*	1797	0.08	0.81	0.4	1.0
rs7174668	15	*MTHFS*	1798	0.21	0.48	0.4	1.0
rs204942	15	*MTHFS*	1798	0.21	0.31	0.4	1.0
rs7594432	2	*DNMT3A*	1798	0.44	0.13	0.4	1.0
rs7217764	17	*PEMT*	1798	0.05	0.51	0.4	1.0
rs11683778	2	*ATIC*	1796	0.03	0.54	0.5	1.0
rs282795	15	*MTHFS*	1797	0.32	0.88	0.5	1.0
rs9001	3	*CHDH*	1793	0.09	0.21	0.5	1.0
rs6495446	15	*MTHFS*	1797	0.26	0.38	0.5	1.0
NA	1	*MTR_01_2_i_order*	1793	0.16	0.81	0.5	1.0
rs632220	11	*FOLH1*	1798	0.09	0.30	0.5	1.0
rs4393531	15	*MTHFS*	1795	0.47	0.63	0.5	1.0
rs6058869	20	*DNMT3B*	1797	0.33	0.76	0.5	1.0
rs1956545	14	*MTHFD1*	1797	0.08	0.17	0.5	1.0
rs1164681	11	*FOLH1*	1798	0.12	0.25	0.5	1.0
rs12462004	19	*DNMT1*	1795	0.08	0.64	0.5	1.0
rs1667627	14	*MTHFD2*	1792	0.47	0.34	0.5	1.0
rs378057	15	*MTHFS*	1797	0.14	0.89	0.5	1.0
rs9282690	3	*ALDH1L1*	1793	0.08	1.00	0.5	1.0
rs770144	15	*MTHFS*	1798	0.20	1.00	0.5	1.0
rs202700	11	*FOLH1*	1734	0.23	0.77	0.5	1.0
rs1870576	15	*MTHFS*	1780	0.46	0.06	0.5	1.0
rs4646410	17	*PEMT*	1795	0.31	0.94	0.5	1.0
rs7929543	11	*FOLH1*	1798	0.07	0.81	0.5	1.0
rs7220132	17	*PEMT*	1798	0.29	0.57	0.5	1.0
rs4819138	21	*SLC19A1*	1797	0.40	0.89	0.5	1.0
rs12903985	15	*MTHFS*	1797	0.29	0.14	0.5	1.0
rs6485991	11	*FOLH1*	1791	0.17	0.81	0.5	1.0
rs6711622	2	*DNMT3A*	1798	0.44	1.00	0.5	1.0
rs2288350	19	*DNMT1*	1798	0.08	0.64	0.5	1.0
rs7177027	15	*MTHFS*	1797	0.24	0.40	0.5	1.0
rs7253062	19	*DNMT1*	1798	0.38	1.00	0.5	1.0
rs7604425	2	*ATIC*	1798	0.35	0.94	0.5	1.0
rs4804125	19	*DNMT1*	1797	0.08	0.64	0.5	1.0
rs1051296	21	*SLC19A1*	1786	0.48	0.10	0.5	1.0
rs4987173	6	*GNMT*	1798	0.50	0.46	0.5	1.0
rs7085104	10	*AS3MT*	1798	0.38	1.00	0.5	1.0

Supplementary Table S4-6 (cont.) Corrected likelihood ratio test p-values of the interaction between LINE-1 methylation and SNPs in one-carbon metabolism pathway and bladder cancer susceptibility genes and in the SBS/EPICURO study

dbSNP (N=524)	Chromosome	Gene	N	MAF	*P* HWE	*P* LRT	Corrected *P* LRT
rs8111085	19	*DNMT1*	1797	0.08	0.64	0.5	1.0
rs2241807	3	*CHDH*	1798	0.42	0.89	0.5	1.0
rs10839229	11	*FOLH1*	1795	0.08	0.35	0.5	1.0
rs11672909	19	*DNMT1*	1797	0.08	0.81	0.5	1.0
rs2236224	14	*MTHFD1*	1798	0.36	0.07	0.5	1.0
rs10165919	2	*ATIC*	1797	0.35	0.88	0.5	1.0
rs660439	11	*FOLH1*	1794	0.23	0.85	0.5	1.0
rs2838977	21	*SLC19A1*	1796	0.40	0.72	0.5	1.0
rs202712	11	*FOLH1*	1796	0.23	0.85	0.5	1.0
rs1081231	15	*MTHFS*	1797	0.17	0.71	0.5	1.0
rs914232	21	*SLC19A1*	1797	0.45	0.17	0.5	1.0
rs2330183	21	*SLC19A1*	1757	0.45	0.05	0.5	1.0
rs11892031	2	*UGT1A*	1798	0.09	1.00	0.5	1.0
rs6119286	20	*DNMT3B*	1798	0.02	0.38	0.5	1.0
rs7117025	11	*FOLH1*	1798	0.09	0.30	0.5	1.0
rs10498514	14	*MTHFD1*	1798	0.03	0.56	0.5	1.0
rs8112895	19	*DNMT1*	1798	0.08	0.81	0.5	1.0
rs1051298	21	*SLC19A1*	1790	0.47	0.17	0.5	1.0
rs10839239	11	*FOLH1*	1795	0.23	0.85	0.5	1.0
rs11701960	21	*SLC19A1*	1797	0.18	0.91	0.5	1.0
rs11040261	11	*FOLH1*	1797	0.09	0.39	0.5	1.0
rs2877078	21	*SLC19A1*	1786	0.40	0.94	0.5	1.0
rs4479310	17	*PEMT*	1798	0.30	0.63	0.5	1.0
rs11607791	11	*FOLH1*	1796	0.07	1.00	0.5	1.0
rs6141813	20	*DNMT3B*	1798	0.14	1.00	0.5	1.0
rs10418707	19	*DNMT1*	1796	0.08	0.64	0.6	1.0
rs12293923	11	*FOLH1*	1792	0.09	0.19	0.6	1.0
rs11158540	14	*MTHFD1*	1798	0.35	0.94	0.6	1.0
rs8074191	17	*PEMT*	1778	0.28	0.93	0.6	1.0
rs3960835	11	*FOLH1*	1798	0.06	0.12	0.6	1.0
rs12913164	15	*MTHFS*	1798	0.08	0.34	0.6	1.0
rs1256107	14	*MTHFD1*	1796	0.49	0.50	0.6	1.0
rs4804494	19	*DNMT1*	1797	0.08	0.64	0.6	1.0
rs1808119	2	*ATIC*	1797	0.19	0.82	0.6	1.0
rs4804490	19	*DNMT1*	1796	0.08	0.64	0.6	1.0
rs7358352	11	*FOLH1*	1797	0.09	0.30	0.6	1.0
rs914231	21	*SLC19A1*	1791	0.45	0.19	0.6	1.0
rs11040263	11	*FOLH1*	1795	0.09	0.19	0.6	1.0
rs598841	11	*FOLH1*	1791	0.08	0.19	0.6	1.0
rs683680	11	*FOLH1*	1797	0.09	0.14	0.6	1.0

Supplementary Table S4-6 (cont.) Corrected likelihood ratio test p-values of the interaction between LINE-1 methylation and SNPs in one-carbon metabolism pathway and bladder cancer susceptibility genes and in the SBS/EPICURO study

dbSNP (N=524)	Chromosome	Gene	N	MAF	*P* HWE	*P* LRT	Corrected *P* LRT
rs11694842	2	*DNMT3A*	1797	0.28	0.36	0.6	1.0
rs10839224	11	*FOLH1*	1798	0.08	0.35	0.6	1.0
rs650826	11	*FOLH1*	1796	0.09	0.14	0.6	1.0
rs920253	3	*CHDH*	1798	0.02	0.08	0.6	1.0
rs2838970	21	*SLC19A1*	1797	0.40	0.78	0.6	1.0
rs35020344	14	*MTHFD1*	1797	0.48	0.42	0.6	1.0
rs6586282	21	*CBS*	1792	0.18	0.16	0.6	1.0
rs16971252	15	*MTHFS*	1798	0.06	0.34	0.6	1.0
rs759920	19	*DNMT1*	1798	0.46	0.79	0.6	1.0
rs4819128	21	*SLC19A1*	1798	0.45	0.13	0.6	1.0
rs6058893	20	*DNMT3B*	1798	0.32	0.49	0.6	1.0
rs7177659	15	*MTHFS*	1796	0.49	0.09	0.6	1.0
rs16853834	2	*ATIC*	1798	0.17	0.71	0.6	1.0
rs3897953	15	*MTHFS*	1798	0.10	0.25	0.6	1.0
rs1256095	14	*MTHFD1*	1782	0.48	0.50	0.6	1.0
rs16971231	15	*MTHFS*	1794	0.05	0.14	0.6	1.0
rs8659	5	*MTRR*	1790	0.35	0.33	0.6	1.0
rs10420338	19	*DNMT1*	1798	0.47	0.64	0.6	1.0
rs2275565	1	*MTR*	1793	0.19	0.66	0.6	1.0
rs2236225	14	*MTHFD1*	1797	0.43	0.24	0.6	1.0
rs17556442	11	*FOLH1*	1792	0.06	0.12	0.6	1.0
rs8034036	15	*MTHFS*	1795	0.11	0.23	0.6	1.0
rs853858	20	*DNMT3B*	1796	0.37	0.19	0.6	1.0
rs4778721	15	*MTHFS*	1798	0.22	0.09	0.6	1.0
rs4778719	15	*MTHFS*	1798	0.22	0.09	0.6	1.0
rs9306264	22	*TCN2*	1797	0.05	1.00	0.6	1.0
rs282772	15	*MTHFS*	1798	0.14	0.25	0.6	1.0
rs445263	15	*MTHFS*	1798	0.29	0.68	0.6	1.0
rs12373907	21	*SLC19A1*	1797	0.38	0.20	0.6	1.0
rs9642880	8	*MYC*	1797	0.44	0.78	0.6	1.0
rs7113251	11	*FOLH1*	1798	0.06	0.12	0.6	1.0
rs9323450	14	*MTHFD1*	1798	0.31	0.69	0.6	1.0
rs2372535	2	*ATIC*	1798	0.14	0.89	0.6	1.0
rs1819409	11	*FOLH1*	1783	0.09	0.10	0.6	1.0
NA	1	*MTHFR_02_ORDER*	1658	0.39	0.33	0.6	1.0
rs1109859	17	*PEMT*	1770	0.18	0.64	0.6	1.0
rs1801131	1	*MTHFR*	1710	0.28	0.34	0.6	1.0
rs8074074	17	*PEMT*	1796	0.30	0.87	0.6	1.0
rs2790	18	*TYMS*	1789	0.20	0.67	0.6	1.0
rs10498034	2	*ATIC*	1798	0.16	0.90	0.6	1.0

Supplementary Table S4-6 (cont.) Corrected likelihood ratio test p-values of the interaction between LINE-1 methylation and SNPs in one-carbon metabolism pathway and bladder cancer susceptibility genes and in the SBS/EPICURO study

dbSNP (N=524)	Chromosome	Gene	N	MAF	*P* HWE	*P* LRT	Corrected *P* LRT
rs17101854	14	*MTHFD1*	1797	0.03	0.53	0.6	1.0
rs2295639	14	*MTHFD1*	1797	0.03	0.54	0.6	1.0
rs376863	15	*MTHFS*	1772	0.50	0.73	0.6	1.0
rs8102137	19	*CCNE1*	1798	0.33	0.13	0.6	1.0
rs2287779	5	*MTRR*	1791	0.03	0.52	0.6	1.0
rs2287780	5	*MTRR*	1793	0.03	0.52	0.6	1.0
rs2281603	14	*MTHFD1*	1798	0.20	0.34	0.6	1.0
rs2228611	19	*DNMT1*	1798	0.46	0.79	0.6	1.0
rs1979276	17	*SHMT1*	1808	0.31	0.94	0.6	1.0
rs1917321	11	*FOLH1*	1790	0.50	0.54	0.6	1.0
rs1650697	5	*DHFR*	1791	0.23	0.06	0.6	1.0
rs9897362	17	*PEMT*	1798	0.06	0.77	0.6	1.0
rs34033751	11	*FOLH1*	1776	0.11	0.23	0.6	1.0
rs12592743	15	*MTHFS*	1798	0.10	0.12	0.6	1.0
rs3783	17	*SHMT1*	1806	0.26	0.30	0.6	1.0
rs7113075	11	*FOLH1*	1797	0.08	0.64	0.7	1.0
rs12900076	15	*MTHFS*	1793	0.08	0.25	0.7	1.0
rs12098986	11	*FOLH1*	1795	0.09	0.20	0.7	1.0
rs17728676	11	*FOLH1*	1797	0.06	0.14	0.7	1.0
rs16853826	2	*ATIC*	1796	0.13	0.88	0.7	1.0
rs1051266	21	*SLC19A1*	1798	0.45	0.20	0.7	1.0
rs767138	21	*SLC19A1*	1795	0.41	0.89	0.7	1.0
rs9977111	21	*SLC19A1*	1750	0.33	0.14	0.7	1.0
rs17284990	15	*MTHFS*	1798	0.21	0.62	0.7	1.0
rs9305012	19	*DNMT1*	1797	0.08	0.64	0.7	1.0
rs1059394	18	*TYMS*	1791	0.31	0.64	0.7	1.0
rs11040353	11	*FOLH1*	1795	0.08	0.64	0.7	1.0
rs7926211	11	*FOLH1*	1794	0.09	0.19	0.7	1.0
rs2290684	19	*DNMT1*	1797	0.46	0.79	0.7	1.0
rs11677670	2	*DNMT3A*	1788	0.18	0.64	0.7	1.0
rs600671	15	*MTHFS*	1797	0.45	0.34	0.7	1.0
rs4610054	2	*ATIC*	1794	0.38	0.67	0.7	1.0
rs1801394	5	*MTRR*	1808	0.49	0.50	0.7	1.0
rs4441015	11	*FOLH1*	1753	0.14	0.40	0.7	1.0
rs4779140	15	*MTHFS*	1797	0.48	0.79	0.7	1.0
rs7085854	10	*AS3MT*	1797	0.22	0.85	0.7	1.0
rs282778	15	*MTHFS*	1798	0.25	0.86	0.7	1.0
rs13401241	2	*DNMT3A*	1798	0.45	0.28	0.7	1.0
rs1473406	15	*MTHFS*	1796	0.15	0.79	0.7	1.0
rs1131603	22	*TCN2*	1797	0.04	0.66	0.7	1.0

Supplementary Table S4-6 (cont.) Corrected likelihood ratio test p-values of the interaction between LINE-1 methylation and SNPs in one-carbon metabolism pathway and bladder cancer susceptibility genes and in the SBS/EPICURO study

dbSNP (N=524)	Chromosome	Gene	N	MAF	*P* HWE	*P* LRT	Corrected *P* LRT
rs2834233	21	*GART*	1797	0.09	0.08	0.7	1.0
rs16971260	15	*MTHFS*	1798	0.04	1.00	0.7	1.0
rs282792	15	*MTHFS*	1798	0.37	0.47	0.7	1.0
rs1846285	11	*FOLH1*	1795	0.16	0.54	0.7	1.0
rs2983733	14	*MTHFD1*	1798	0.44	0.68	0.7	1.0
rs4818789	21	*SLC19A1*	1798	0.25	0.06	0.7	1.0
rs12482067	21	*GART*	1798	0.02	0.24	0.7	1.0
rs6413471	1	*CTH*	1793	0.05	0.72	0.7	1.0
rs9836592	3	*CHDH*	1798	0.32	0.14	0.7	1.0
rs7934591	11	*FOLH1*	1798	0.08	0.81	0.7	1.0
rs898436	15	*MTHFS*	1793	0.45	0.37	0.7	1.0
rs12222221	11	*FOLH1*	1798	0.08	1.00	0.7	1.0
rs11040390	11	*FOLH1*	1798	0.08	1.00	0.7	1.0
rs4819130	21	*SLC19A1*	1794	0.45	0.15	0.7	1.0
rs372447	15	*MTHFS*	1798	0.38	0.77	0.7	1.0
rs2586182	15	*MTHFS*	1798	0.14	0.78	0.7	1.0
rs202718	11	*FOLH1*	1798	0.15	0.79	0.7	1.0
rs1404772	2	*ATIC*	1798	0.08	0.34	0.7	1.0
rs699517	18	*TYMS*	1791	0.31	0.64	0.7	1.0
rs8068641	17	*PEMT*	1792	0.11	0.62	0.7	1.0
rs17291414	19	*DNMT1*	1798	0.28	0.73	0.7	1.0
rs11191457	10	*AS3MT*	1794	0.22	1.00	0.7	1.0
rs401681	5	*TERT-CLPTM1L*	1798	0.46	0.84	0.7	1.0
rs3788200	21	*SLC19A1*	1798	0.45	0.31	0.7	1.0
rs4779141	15	*MTHFS*	1793	0.34	0.55	0.7	1.0
rs11627387	14	*MTHFD1*	1798	0.30	0.81	0.7	1.0
rs8003379	14	*MTHFD1*	1796	0.25	0.78	0.7	1.0
rs4646383	17	*PEMT*	1797	0.09	0.53	0.7	1.0
rs13317328	3	*CHDH*	1798	0.09	0.06	0.7	1.0
rs7587636	2	*DNMT3A*	1798	0.45	0.25	0.7	1.0
rs3177999	21	*SLC19A1*	1790	0.46	0.17	0.7	1.0
rs202676	11	*FOLH1*	1788	0.17	0.72	0.7	1.0
rs8129350	21	*SLC19A1*	1797	0.34	0.76	0.7	1.0
rs2983736	14	*MTHFD1*	1793	0.44	0.73	0.7	1.0
rs3818239	14	*MTHFD1*	1787	0.13	0.66	0.7	1.0
rs7946	17	*PEMT*	1798	0.30	0.52	0.7	1.0
rs1464864	2	*ATIC*	1798	0.30	0.81	0.7	1.0
rs5753231	22	*TCN2*	1797	0.16	0.62	0.7	1.0
rs3893384	15	*MTHFS*	1798	0.42	0.44	0.7	1.0
rs12999687	2	*DNMT3A*	1795	0.45	0.68	0.7	1.0

Supplementary Table S4-6 (cont.) Corrected likelihood ratio test p-values of the interaction between LINE-1 methylation and SNPs in one-carbon metabolism pathway and bladder cancer susceptibility genes and in the SBS/EPICURO study

dbSNP (N=524)	Chromosome	Gene	N	MAF	*P* HWE	*P* LRT	Corrected *P* LRT
rs6087988	20	*DNMT3B*	1798	0.19	1.00	0.7	1.0
rs9974061	21	*SLC19A1*	1798	0.18	0.82	0.7	1.0
rs11040432	11	*FOLH1*	1797	0.08	1.00	0.7	1.0
rs6722613	2	*DNMT3A*	1798	0.40	0.89	0.7	1.0
rs648372	11	*FOLH1*	1772	0.16	0.46	0.8	1.0
rs234706	21	*CBS*	1793	0.33	0.36	0.8	1.0
rs437302	20	*DNMT3B*	1797	0.08	1.00	0.8	1.0
rs12591436	15	*MTHFS*	1798	0.35	0.55	0.8	1.0
rs8128050	21	*SLC19A1*	1794	0.34	0.94	0.8	1.0
rs1888530	21	*SLC19A1*	1760	0.47	0.08	0.8	1.0
rs8128676	21	*SLC19A1*	1754	0.22	0.92	0.8	1.0
rs7951180	11	*FOLH1*	1778	0.17	0.55	0.8	1.0
rs3772078	2	*ATIC*	1797	0.20	0.29	0.8	1.0
rs11158538	14	*MTHFD1*	1795	0.45	0.78	0.8	1.0
rs2066470	1	*MTHFR*	1788	0.08	0.39	0.8	1.0
rs16853782	2	*ATIC*	1798	0.20	0.34	0.8	1.0
rs679470	11	*FOLH1*	1797	0.17	0.63	0.8	1.0
rs11158542	14	*MTHFD1*	1798	0.30	0.81	0.8	1.0
rs2586154	15	*MTHFS*	1798	0.14	0.89	0.8	1.0
rs944422	21	*SLC19A1*	1791	0.35	0.76	0.8	1.0
rs1888533	21	*SLC19A1*	1797	0.48	0.42	0.8	1.0
rs2424932	20	*DNMT3B*	1797	0.43	0.15	0.8	1.0
rs8015278	14	*MTHFD1*	1798	0.07	0.18	0.8	1.0
rs12438477	15	*MTHFS*	1797	0.36	0.61	0.8	1.0
rs1076991	14	*MTHFD1*	1798	0.45	0.63	0.8	1.0
rs12898642	15	*MTHFS*	1798	0.43	0.34	0.8	1.0
rs3821353	2	*ATIC*	1798	0.20	0.17	0.8	1.0
rs11687225	2	*ATIC*	1797	0.40	0.72	0.8	1.0
rs12884767	14	*MTHFD1*	1798	0.04	0.14	0.8	1.0
rs8128681	21	*SLC19A1*	1798	0.33	0.94	0.8	1.0
rs685487	15	*MTHFS*	1798	0.36	0.34	0.8	1.0
rs5749131	22	*TCN2*	1798	0.42	0.13	0.8	1.0
rs282802	15	*MTHFS*	1798	0.29	0.09	0.8	1.0
rs1256114	14	*MTHFD1*	1797	0.11	0.86	0.8	1.0
rs35709834	5	*DHFR*	1793	0.04	0.62	0.8	1.0
rs9910747	17	*PEMT*	1798	0.07	0.47	0.8	1.0
rs11085720	19	*DNMT1*	1798	0.41	0.44	0.8	1.0
rs7144437	14	*MTHFD1*	1797	0.07	0.10	0.8	1.0
rs13036246	2	*DNMT3A*	1797	0.48	0.08	0.8	1.0
rs3740392	10	*AS3MT*	1794	0.29	0.41	0.8	1.0

Supplementary Table S4-6 (cont.) Corrected likelihood ratio test p-values of the interaction between LINE-1 methylation and SNPs in one-carbon metabolism pathway and bladder cancer susceptibility genes and in the SBS/EPICURO study

dbSNP (N=524)	Chromosome	Gene	N	MAF	*P* HWE	*P* LRT	Corrected *P* LRT
rs1020697	17	*PEMT*	1770	0.10	0.35	0.8	1.0
rs2865908	11	*FOLH1*	1798	0.18	0.50	0.8	1.0
rs6573559	14	*MTHFD1*	1798	0.30	0.94	0.8	1.0
rs7102641	11	*FOLH1*	1793	0.08	0.47	0.8	1.0
rs3774616	3	*CHDH*	1798	0.04	1.00	0.8	1.0
rs12613	21	*CBS*	1793	0.09	0.83	0.8	1.0
rs1465825	2	*DNMT3A*	1797	0.27	1.00	0.8	1.0
rs1023159	21	*SLC19A1*	1796	0.42	0.78	0.8	1.0
rs1979277	17	*SHMT1*	1809	0.27	0.87	0.8	1.0
rs11040106	11	*FOLH1*	1787	0.36	0.66	0.9	1.0
rs12148881	15	*MTHFS*	1797	0.26	0.19	0.9	1.0
rs16971249	15	*MTHFS*	1798	0.08	0.09	0.9	1.0
rs2424908	20	*DNMT3B*	1798	0.17	0.40	0.9	1.0
rs734693	2	*DNMT3A*	1795	0.29	0.51	0.9	1.0
rs2838961	21	*SLC19A1*	1797	0.34	0.94	0.9	1.0
rs12453139	17	*PEMT*	1797	0.26	0.66	0.9	1.0
NA	1	*MTHFR_02_2_i_order*	1793	0.39	0.35	0.9	1.0
rs1801133	1	*MTHFR*	1793	0.39	0.35	0.9	1.0
rs17745484	2	*DNMT3A*	1797	0.35	0.30	0.9	1.0
rs4902278	14	*MTHFD1*	1791	0.07	0.18	0.9	1.0
rs2838958	21	*SLC19A1*	1794	0.46	0.63	0.9	1.0
rs8018032	14	*MTHFD1*	1798	0.45	0.95	0.9	1.0
rs10839210	11	*FOLH1*	1796	0.21	0.18	0.9	1.0
rs1046778	10	*AS3MT*	1798	0.32	0.70	0.9	1.0
rs12987326	2	*DNMT3A*	1798	0.37	0.47	0.9	1.0
rs11681447	2	*DNMT3A*	1796	0.29	0.62	0.9	1.0
rs2987969	14	*MTHFD1*	1797	0.45	0.95	0.9	1.0
rs2834232	21	*GART*	1797	0.26	0.73	0.9	1.0
rs8971	21	*GART*	1795	0.26	0.73	0.9	1.0
rs2289093	2	*DNMT3A*	1798	0.29	0.62	0.9	1.0
rs1847638	11	*FOLH1*	1729	0.21	0.47	0.9	1.0
rs17279885	15	*MTHFS*	1798	0.20	0.67	0.9	1.0
rs2834231	21	*GART*	1798	0.26	0.73	0.9	1.0
rs8011839	14	*MTHFD1*	1798	0.17	0.72	0.9	1.0
rs1256142	14	*MTHFD1*	1798	0.44	0.10	0.9	1.0
rs8923	15	*MTHFS*	1798	0.08	0.15	0.9	1.0
rs10460566	2	*DNMT3A*	1798	0.27	0.86	0.9	1.0
rs6445606	3	*CHDH*	1798	0.29	0.10	0.9	1.0
rs11852515	15	*MTHFS*	1798	0.11	0.74	0.9	1.0
rs10769558	11	*FOLH1*	1797	0.21	0.18	0.9	1.0

Supplementary Table S4-6 (cont.) Corrected likelihood ratio test p-values of the interaction between LINE-1 methylation and SNPs in one-carbon metabolism pathway and bladder cancer susceptibility genes and in the SBS/EPICURO study

dbSNP (N=524)	Chromosome	Gene	N	MAF	*P* HWE	*P* LRT	Corrected *P* LRT
rs2236222	14	*MTHFD1*	1798	0.10	0.27	0.9	1.0
rs6517178	21	*GART*	1798	0.40	0.72	0.9	1.0
rs12627639	21	*SLC19A1*	1797	0.21	0.68	0.9	1.0
rs17279753	15	*MTHFS*	1798	0.19	0.51	0.9	1.0
rs12614943	2	*ATIC*	1798	0.27	0.73	0.9	1.0
rs3783728	14	*MTHFD1*	1798	0.08	0.64	0.9	1.0
rs8129445	21	*SLC19A1*	1798	0.32	0.70	0.9	1.0
rs12416687	10	*AS3MT*	1797	0.27	0.15	0.9	1.0
rs4094478	11	*FOLH1*	1770	0.20	0.34	0.9	1.0
rs2154583	21	*GART*	1791	0.40	0.67	0.9	1.0
rs1809986	11	*FOLH1*	1798	0.36	0.38	0.9	1.0
rs4817579	21	*GART*	1798	0.34	0.15	0.9	1.0
rs8003567	14	*MTHFD1*	1798	0.11	0.47	0.9	1.0
rs7283354	21	*GART*	1798	0.34	0.15	0.9	1.0
rs749130	2	*DNMT3A*	1798	0.45	0.37	0.9	1.0
rs35918857	19	*DNMT1*	1797	0.02	0.25	0.9	1.0
rs16999714	19	*DNMT1*	1796	0.21	0.26	0.9	1.0
rs8081810	17	*PEMT*	1797	0.20	0.92	0.9	1.0
rs7279305	21	*SLC19A1*	1797	0.35	1.00	0.9	1.0
rs7120743	11	*FOLH1*	1774	0.36	0.77	0.9	1.0
rs1404774	2	*ATIC*	1784	0.22	0.42	1.0	1.0
rs559062	1	*CTH*	1793	0.22	0.85	1.0	1.0
rs4817577	21	*GART*	1797	0.34	0.20	1.0	1.0
rs3755817	3	*CHDH*	1797	0.30	0.42	1.0	1.0
rs4911263	20	*DNMT3B*	1798	0.32	1.00	1.0	1.0
rs17751556	14	*MTHFD1*	1798	0.08	0.65	1.0	1.0
rs12910340	15	*MTHFS*	1798	0.42	0.73	1.0	1.0
rs2838969	21	*SLC19A1*	1798	0.07	0.79	1.0	1.0
rs2696923	11	*FOLH1*	1798	0.21	0.12	1.0	1.0
rs8041943	15	*MTHFS*	1794	0.41	0.37	1.0	1.0
rs11629135	14	*MTHFD1*	1797	0.11	0.38	1.0	1.0
rs11627525	14	*MTHFD1*	1798	0.11	0.08	1.0	1.0
rs865646	5	*DHFR*	1736	0.36	0.55	1.0	1.0
rs2834235	21	*GART*	1797	0.39	0.67	1.0	1.0
rs2696935	11	*FOLH1*	1798	0.21	0.12	1.0	1.0
rs663465	1	*CTH_01*	1792	0.42	0.36	1.0	1.0
rs8019804	14	*MTHFD1*	1798	0.07	1.00	1.0	1.0
rs6801605	3	*CHDH*	1798	0.37	1.00	1.0	1.0
rs3800292	6	*GNMT*	1798	0.06	0.57	1.0	1.0
rs2987981	14	*MTHFD1*	1798	0.26	1.00	1.0	1.0

Supplementary Table S4-6 (cont.) Corrected likelihood ratio test p-values of the interaction between LINE-1 methylation and SNPs in one-carbon metabolism pathway and bladder cancer susceptibility genes and in the SBS/EPICURO study

dbSNP (N=524)	Chromosome	Gene	N	MAF	*P* HWE	*P* LRT	Corrected *P* LRT
rs11658944	17	*PEMT*	1797	0.05	0.49	1.0	1.0
rs617219	5	*BHMT*	1790	0.32	0.94	1.0	1.0
rs567754	5	*BHMT*	1792	0.29	0.74	1.0	1.0
rs7759302	6	*GNMT*	1798	0.06	0.57	1.0	1.0

Supplementary Table S4-6 (cont.) Corrected likelihood ratio test p-values of the interaction between LINE-1 methylation and SNPs in one-carbon metabolism pathway and bladder cancer susceptibility genes and in the SBS/EPICURO study

SNPs modeled in codominant mode of inheritance							
dbSNP (N=457)	Chromosome	Gene	N	MAF	*P* HWE	*P* LRT	Corrected *P* LRT
rs7215833	17	*PEMT*	1798	0.36	0.51	0.0001	0.08
rs2124344	17	*PEMT*	1797	0.36	0.77	0.0001	0.08
rs4646340	17	*PEMT*	1798	0.37	0.51	0.0002	0.1
rs4646341	17	*PEMT*	1795	0.37	0.56	0.0004	0.2
rs4646350	17	*PEMT*	1798	0.36	0.83	0.0004	0.2
rs7604984	2	*ATIC*	1798	0.40	0.62	0.004	0.9
rs4646359	17	*PEMT*	1798	0.46	0.15	0.004	0.9
rs10498036	2	*ATIC*	1798	0.40	0.57	0.005	0.9
rs11892429	2	*ATIC*	1798	0.29	1.00	0.007	1.0
rs10197559	2	*ATIC*	1788	0.29	0.87	0.007	1.0
rs11683424	2	*DNMT3A*	1798	0.12	0.20	0.008	1.0
rs9890064	17	*PEMT*	1798	0.43	0.41	0.009	1.0
rs10179873	2	*ATIC*	1798	0.30	0.63	0.009	1.0
rs798766	4	*TMEM129-TACC3-FGFR3*	1798	0.18	0.21	0.009	1.0
rs10197653	2	*ATIC*	1798	0.29	0.74	0.01	1.0
rs4673991	2	*ATIC*	1797	0.32	0.31	0.01	1.0
rs4673993	2	*ATIC*	1798	0.32	0.31	0.01	1.0
rs4672768	2	*ATIC*	1794	0.32	0.31	0.01	1.0
rs11855092	15	*MTHFS*	1798	0.24	0.31	0.01	1.0
rs1983462	2	*ATIC*	1798	0.31	0.47	0.02	1.0
rs7560488	2	*DNMT3A*	1731	0.48	0.15	0.02	1.0
rs6706415	2	*ATIC*	1798	0.31	0.81	0.02	1.0
rs1077965	15	*MTHFS*	1797	0.41	0.58	0.02	1.0
rs435689	15	*MTHFS*	1798	0.49	0.50	0.02	1.0
rs1880580	15	*MTHFS*	1798	0.31	0.38	0.02	1.0
rs7604425	2	*ATIC*	1798	0.35	0.94	0.02	1.0
rs4924922	17	*PEMT*	1798	0.37	0.43	0.02	1.0
rs282814	15	*MTHFS*	1798	0.22	0.84	0.02	1.0
rs7581217	2	*DNMT3A*	1798	0.39	0.62	0.02	1.0
rs6495441	15	*MTHFS*	1798	0.25	0.93	0.02	1.0
rs10165919	2	*ATIC*	1797	0.35	0.88	0.02	1.0
rs12903985	15	*MTHFS*	1797	0.29	0.14	0.03	1.0
rs204942	15	*MTHFS*	1798	0.21	0.31	0.03	1.0
rs2733106	15	*MTHFS*	1793	0.15	0.69	0.03	1.0
rs11656215	17	*PEMT*	1798	0.46	0.20	0.03	1.0
rs7174668	15	*MTHFS*	1798	0.21	0.48	0.04	1.0
rs4673965	2	*ATIC*	1798	0.40	0.94	0.04	1.0
rs4646404	17	*PEMT*	1792	0.35	0.26	0.04	1.0
rs4244599	17	*PEMT*	1774	0.47	0.34	0.05	1.0
rs2586153	15	*MTHFS*	1777	0.15	0.59	0.05	1.0

Supplementary Table S4-6 (cont.) Corrected likelihood ratio test p-values of the interaction between LINE-1 methylation and SNPs in one-carbon metabolism pathway and bladder cancer susceptibility genes and in the SBS/EPICURO study

dbSNP (N=457)	Chromosome	Gene	N	MAF	*P* HWE	*P* LRT	Corrected *P* LRT
rs4925048	17	*PEMT*	1797	0.10	0.17	0.06	1.0
rs4393531	15	*MTHFS*	1795	0.47	0.63	0.06	1.0
rs11869600	17	*PEMT*	1796	0.37	0.38	0.07	1.0
rs7563206	2	*ATIC*	1796	0.47	0.84	0.07	1.0
rs12997662	2	*ATIC*	1798	0.34	0.26	0.07	1.0
rs282795	15	*MTHFS*	1797	0.32	0.88	0.08	1.0
rs11871738	17	*PEMT*	1798	0.38	0.67	0.08	1.0
rs4646385	17	*PEMT*	1798	0.45	0.37	0.08	1.0
rs4817580	21	*GART*	1797	0.10	0.70	0.09	1.0
rs1880586	2	*ATIC*	1797	0.47	0.89	0.09	1.0
rs750546	17	*PEMT*	1773	0.45	0.19	0.09	1.0
rs4610054	2	*ATIC*	1794	0.38	0.67	0.10	1.0
rs12905663	15	*MTHFS*	1788	0.29	0.80	0.1	1.0
rs10509760	10	*AS3MT*	1798	0.13	0.88	0.1	1.0
rs4646344	17	*PEMT*	1798	0.46	0.45	0.1	1.0
rs3740394	10	*AS3MT*	1797	0.13	1.00	0.1	1.0
rs17209637	15	*MTHFS*	1794	0.26	0.79	0.1	1.0
rs944422	21	*SLC19A1*	1791	0.35	0.76	0.1	1.0
rs4819130	21	*SLC19A1*	1794	0.45	0.15	0.1	1.0
rs2838961	21	*SLC19A1*	1797	0.34	0.94	0.1	1.0
rs6713377	2	*DNMT3A*	1797	0.47	0.89	0.1	1.0
rs11040421	11	*FOLH1*	1798	0.14	0.78	0.1	1.0
rs3760188	17	*PEMT*	1798	0.46	0.38	0.1	1.0
rs10418	22	*TCN2*	1772	0.21	0.41	0.1	1.0
rs7575625	2	*DNMT3A*	1797	0.47	0.89	0.1	1.0
NA	1	*MTHFR_02_ORDER*	1658	0.39	0.33	0.1	1.0
rs6749992	2	*DNMT3A*	1798	0.47	0.79	0.1	1.0
rs914238	21	*SLC19A1*	1798	0.49	0.89	0.1	1.0
rs8129350	21	*SLC19A1*	1797	0.34	0.76	0.1	1.0
rs1604503	15	*MTHFS*	1798	0.15	0.59	0.2	1.0
rs10380	5	*MTRR*	1793	0.10	0.56	0.2	1.0
rs4817577	21	*GART*	1797	0.34	0.20	0.2	1.0
rs17824591	14	*MTHFD1*	1796	0.23	0.63	0.2	1.0
rs1014971	22	*CBX6 APOBEC3A*	1797	0.30	0.23	0.2	1.0
rs8102137	19	*CCNE1*	1798	0.33	0.13	0.2	1.0
rs6058897	20	*DNMT3B*	1798	0.44	0.17	0.2	1.0
rs7279305	21	*SLC19A1*	1797	0.35	1.00	0.2	1.0
rs7283354	21	*GART*	1798	0.34	0.15	0.2	1.0
rs1051266	21	*SLC19A1*	1798	0.45	0.20	0.2	1.0
rs13036246	2	*DNMT3A*	1797	0.48	0.08	0.2	1.0

Supplementary Table S4-6 (cont.) Corrected likelihood ratio test p-values of the interaction between LINE-1 methylation and SNPs in one-carbon metabolism pathway and bladder cancer susceptibility genes and in the SBS/EPICURO study

dbSNP (N=457)	Chromosome	Gene	N	MAF	*P* HWE	*P* LRT	Corrected *P* LRT
rs12898670	15	*MTHFS*	1796	0.34	0.70	0.2	1.0
rs1074390	15	*MTHFS*	1798	0.38	0.77	0.2	1.0
rs1380642	15	*MTHFS*	1798	0.18	0.57	0.2	1.0
rs11191439	10	*AS3MT*	1795	0.12	0.75	0.2	1.0
rs2866358	11	*FOLH1*	1781	0.38	0.35	0.2	1.0
rs11892646	2	*DNMT3A*	1797	0.11	0.87	0.2	1.0
rs1460177	15	*MTHFS*	1797	0.08	0.37	0.2	1.0
rs4817579	21	*GART*	1798	0.34	0.15	0.2	1.0
rs9001	3	*CHDH*	1793	0.09	0.21	0.2	1.0
rs7605753	2	*DNMT3A*	1797	0.47	0.68	0.2	1.0
rs7220132	17	*PEMT*	1798	0.29	0.57	0.2	1.0
rs3177999	21	*SLC19A1*	1790	0.46	0.17	0.2	1.0
rs740234	22	*TCN2*	1798	0.23	0.39	0.2	1.0
rs2733103	15	*MTHFS*	1797	0.15	0.51	0.2	1.0
rs9835128	3	*CHDH*	1797	0.16	0.38	0.2	1.0
rs1495741	8	*NAT2*	1798	0.24	1.00	0.2	1.0
rs3788200	21	*SLC19A1*	1798	0.45	0.31	0.2	1.0
rs8034036	15	*MTHFS*	1795	0.11	0.23	0.2	1.0
rs1109859	17	*PEMT*	1770	0.18	0.64	0.2	1.0
rs4144700	11	*FOLH1*	1798	0.38	0.47	0.2	1.0
rs10839295	11	*FOLH1*	1797	0.40	0.48	0.2	1.0
rs1814175	11	*FOLH1*	1793	0.40	0.40	0.2	1.0
rs11852515	15	*MTHFS*	1798	0.11	0.74	0.2	1.0
rs12910340	15	*MTHFS*	1798	0.42	0.73	0.2	1.0
rs16971253	15	*MTHFS*	1797	0.10	1.00	0.2	1.0
rs7583409	2	*DNMT3A*	1795	0.36	0.38	0.2	1.0
rs1917311	11	*FOLH1*	1753	0.40	0.72	0.2	1.0
rs853858	20	*DNMT3B*	1796	0.37	0.19	0.2	1.0
rs7111215	11	*FOLH1*	1774	0.40	0.52	0.2	1.0
rs9606756	22	*TCN2*	1798	0.12	0.87	0.2	1.0
rs3862350	11	*FOLH1*	1765	0.40	0.15	0.2	1.0
rs5753231	22	*TCN2*	1797	0.16	0.62	0.2	1.0
rs8128050	21	*SLC19A1*	1794	0.34	0.94	0.2	1.0
rs1164685	11	*FOLH1*	1793	0.38	0.47	0.3	1.0
rs4779141	15	*MTHFS*	1793	0.34	0.55	0.3	1.0
rs582172	15	*MTHFS*	1798	0.42	0.45	0.3	1.0
rs2241807	3	*CHDH*	1798	0.42	0.89	0.3	1.0
rs1127717	3	*ALDH1L1*	1793	0.24	1.00	0.3	1.0
rs7586294	2	*DNMT3A*	1797	0.47	0.73	0.3	1.0
rs2424913	20	*DNMT3B*	1797	0.37	0.39	0.3	1.0

Supplementary Table S4-6 (cont.) Corrected likelihood ratio test p-values of the interaction between LINE-1 methylation and SNPs in one-carbon metabolism pathway and bladder cancer susceptibility genes and in the SBS/EPICURO study

dbSNP (N=457)	Chromosome	Gene	N	MAF	*P* HWE	*P* LRT	Corrected *P* LRT
rs4531931	2	*ATIC*	1796	0.31	0.23	0.3	1.0
rs9332	5	*MTRR*	1793	0.12	0.34	0.3	1.0
rs710521	3	*TP63*	1798	0.26	0.93	0.3	1.0
rs3755817	3	*CHDH*	1797	0.30	0.42	0.3	1.0
rs4476347	2	*ATIC*	1798	0.25	0.28	0.3	1.0
rs166868	15	*MTHFS*	1796	0.37	0.94	0.3	1.0
rs282772	15	*MTHFS*	1798	0.14	0.25	0.3	1.0
rs282776	15	*MTHFS*	1798	0.36	0.47	0.3	1.0
rs6518253	21	*SLC19A1*	1797	0.46	0.63	0.3	1.0
rs588458	11	*FOLH1*	1772	0.38	0.52	0.3	1.0
rs748196	17	*PEMT*	1795	0.44	0.89	0.3	1.0
rs2838965	21	*SLC19A1*	1792	0.42	0.33	0.3	1.0
rs10839296	11	*FOLH1*	1777	0.25	0.65	0.3	1.0
rs445263	15	*MTHFS*	1798	0.29	0.68	0.3	1.0
rs7107178	11	*FOLH1*	1796	0.25	0.86	0.3	1.0
rs1809986	11	*FOLH1*	1798	0.36	0.38	0.3	1.0
rs2838973	21	*SLC19A1*	1798	0.20	0.92	0.3	1.0
rs2424906	20	*DNMT3B*	1798	0.37	0.28	0.3	1.0
rs8129445	21	*SLC19A1*	1798	0.32	0.70	0.3	1.0
rs1650697	5	*DHFR*	1791	0.23	0.06	0.3	1.0
rs13427202	2	*DNMT3A*	1797	0.47	0.68	0.3	1.0
NA	1	*MTHFR_02_2_i_order*	1793	0.39	0.35	0.3	1.0
rs1801133	1	*MTHFR*	1793	0.39	0.35	0.3	1.0
rs7085104	10	*AS3MT*	1798	0.38	1.00	0.3	1.0
rs7111711	11	*FOLH1*	1798	0.25	0.86	0.3	1.0
rs17211644	15	*MTHFS*	1798	0.10	0.72	0.3	1.0
rs1802059	5	*MTRR*	1792	0.36	0.60	0.3	1.0
rs2183601	21	*SLC19A1*	1797	0.20	0.92	0.3	1.0
rs7951180	11	*FOLH1*	1778	0.17	0.55	0.4	1.0
rs3783	17	*SHMT1*	1806	0.26	0.30	0.4	1.0
rs1801131	1	*MTHFR*	1710	0.28	0.34	0.4	1.0
rs3862342	11	*FOLH1*	1795	0.28	0.93	0.4	1.0
rs2330183	21	*SLC19A1*	1757	0.45	0.05	0.4	1.0
rs8074074	17	*PEMT*	1796	0.30	0.87	0.4	1.0
rs914231	21	*SLC19A1*	1791	0.45	0.19	0.4	1.0
rs914232	21	*SLC19A1*	1797	0.45	0.17	0.4	1.0
rs1059394	18	*TYMS*	1791	0.31	0.64	0.4	1.0
rs1081231	15	*MTHFS*	1797	0.17	0.71	0.4	1.0
rs6058891	20	*DNMT3B*	1796	0.39	0.32	0.4	1.0
rs2838958	21	*SLC19A1*	1794	0.46	0.63	0.4	1.0

Supplementary Table S4-6 (cont.) Corrected likelihood ratio test p-values of the interaction between LINE-1 methylation and SNPs in one-carbon metabolism pathway and bladder cancer susceptibility genes and in the SBS/EPICURO study

dbSNP (N=457)	Chromosome	Gene	N	MAF	*P* HWE	*P* LRT	Corrected *P* LRT
rs2116940	19	*DNMT1*	1797	0.08	0.64	0.4	1.0
rs4819128	21	*SLC19A1*	1798	0.45	0.13	0.4	1.0
rs6586282	21	*CBS*	1792	0.18	0.16	0.4	1.0
rs1950902	14	*MTHFD1*	1798	0.15	1.00	0.4	1.0
rs7120743	11	*FOLH1*	1774	0.36	0.77	0.4	1.0
rs2150460	21	*SLC19A1*	1798	0.20	1.00	0.4	1.0
rs2424921	20	*DNMT3B*	1798	0.39	0.32	0.4	1.0
rs897453	17	*PEMT*	1790	0.47	0.54	0.4	1.0
rs2424914	20	*DNMT3B*	1798	0.39	0.39	0.4	1.0
rs6058883	20	*DNMT3B*	1797	0.39	0.36	0.4	1.0
rs4911263	20	*DNMT3B*	1798	0.32	1.00	0.4	1.0
rs3893384	15	*MTHFS*	1798	0.42	0.44	0.4	1.0
rs13317328	3	*CHDH*	1798	0.09	0.06	0.4	1.0
rs699517	18	*TYMS*	1791	0.31	0.64	0.4	1.0
rs2283873	22	*TCN2*	1778	0.03	0.49	0.4	1.0
rs17745484	2	*DNMT3A*	1797	0.35	0.30	0.4	1.0
rs34048824	2	*DNMT3A*	1797	0.51	0.74	0.4	1.0
rs2294008	8	*PSCA*	1798	0.46	0.73	0.4	1.0
rs8081810	17	*PEMT*	1797	0.20	0.92	0.4	1.0
rs4434082	21	*SLC19A1*	1797	0.20	1.00	0.4	1.0
rs4479310	17	*PEMT*	1798	0.30	0.63	0.4	1.0
rs6760069	2	*ATIC*	1797	0.15	0.35	0.4	1.0
rs11040106	11	*FOLH1*	1787	0.36	0.66	0.4	1.0
rs11040416	11	*FOLH1*	1798	0.42	0.58	0.4	1.0
rs9976878	21	*SLC19A1*	1797	0.20	1.00	0.4	1.0
rs2275565	1	*MTR*	1793	0.19	0.66	0.4	1.0
rs6141813	20	*DNMT3B*	1798	0.14	1.00	0.4	1.0
rs4646410	17	*PEMT*	1795	0.31	0.94	0.4	1.0
rs12884767	14	*MTHFD1*	1798	0.04	0.14	0.4	1.0
rs648372	11	*FOLH1*	1772	0.16	0.46	0.4	1.0
rs10163099	15	*MTHFS*	1792	0.26	0.48	0.4	1.0
rs9974061	21	*SLC19A1*	1798	0.18	0.82	0.4	1.0
rs12462004	19	*DNMT1*	1795	0.08	0.64	0.5	1.0
rs9306139	21	*SLC19A1*	1796	0.20	1.00	0.5	1.0
rs5749135	22	*TCN2*	1798	0.43	0.07	0.5	1.0
rs9462856	6	*GNMT*	1798	0.42	0.53	0.5	1.0
rs9789571	2	*ATIC*	1798	0.42	0.89	0.5	1.0
rs2424922	20	*DNMT3B*	1796	0.39	0.29	0.5	1.0
rs12453139	17	*PEMT*	1797	0.26	0.66	0.5	1.0
rs7946	17	*PEMT*	1798	0.30	0.52	0.5	1.0

Supplementary Table S4-6 (cont.) Corrected likelihood ratio test p-values of the interaction between LINE-1 methylation and SNPs in one-carbon metabolism pathway and bladder cancer susceptibility genes and in the SBS/EPICURO study

dbSNP (N=457)	Chromosome	Gene	N	MAF	*P* HWE	*P* LRT	Corrected *P* LRT
rs10932605	2	*ATIC*	1798	0.14	0.39	0.5	1.0
rs6757920	2	*ATIC*	1794	0.48	0.09	0.5	1.0
rs2424928	20	*DNMT3B*	1798	0.39	0.26	0.5	1.0
rs2834233	21	*GART*	1797	0.09	0.08	0.5	1.0
rs1917321	11	*FOLH1*	1790	0.50	0.54	0.5	1.0
rs2275566	1	*MTR*	1793	0.41	0.53	0.5	1.0
rs4804125	19	*DNMT1*	1797	0.08	0.64	0.5	1.0
rs378057	15	*MTHFS*	1797	0.14	0.89	0.5	1.0
rs679470	11	*FOLH1*	1797	0.17	0.63	0.5	1.0
rs2288350	19	*DNMT1*	1798	0.08	0.64	0.5	1.0
rs8111085	19	*DNMT1*	1797	0.08	0.64	0.5	1.0
rs443394	15	*MTHFS*	1798	0.42	0.30	0.5	1.0
rs11672909	19	*DNMT1*	1797	0.08	0.81	0.5	1.0
rs1956545	14	*MTHFD1*	1797	0.08	0.17	0.5	1.0
rs12591436	15	*MTHFS*	1798	0.35	0.55	0.5	1.0
rs2301955	22	*TCN2*	1794	0.43	0.10	0.5	1.0
rs7124266	11	*FOLH1*	1798	0.30	0.94	0.5	1.0
rs4532960	10	*AS3MT*	1797	0.44	0.89	0.5	1.0
rs3785499	17	*PEMT*	1798	0.48	0.54	0.5	1.0
rs8112895	19	*DNMT1*	1798	0.08	0.81	0.5	1.0
rs10748835	10	*AS3MT*	1798	0.44	0.89	0.5	1.0
rs202676	11	*FOLH1*	1788	0.17	0.72	0.5	1.0
rs12797843	11	*FOLH1*	1798	0.13	0.37	0.5	1.0
rs10948059	6	*GNMT*	1778	0.49	0.54	0.5	1.0
rs6495446	15	*MTHFS*	1797	0.26	0.38	0.5	1.0
rs2267163	22	*TCN2*	1794	0.43	0.06	0.5	1.0
rs8074191	17	*PEMT*	1778	0.28	0.93	0.5	1.0
rs10418707	19	*DNMT1*	1796	0.08	0.64	0.5	1.0
rs4646364	17	*PEMT*	1795	0.03	0.15	0.5	1.0
rs6119286	20	*DNMT3B*	1798	0.02	0.38	0.5	1.0
rs12797853	11	*FOLH1*	1794	0.13	0.46	0.5	1.0
rs4804494	19	*DNMT1*	1797	0.08	0.64	0.5	1.0
rs4804490	19	*DNMT1*	1796	0.08	0.64	0.5	1.0
rs4819138	21	*SLC19A1*	1797	0.40	0.89	0.5	1.0
rs1256112	14	*MTHFD1*	1798	0.40	0.29	0.5	1.0
rs660439	11	*FOLH1*	1794	0.23	0.85	0.5	1.0
rs4911108	20	*DNMT3B*	1793	0.28	0.87	0.5	1.0
rs2281603	14	*MTHFD1*	1798	0.20	0.34	0.6	1.0
rs202718	11	*FOLH1*	1798	0.15	0.79	0.6	1.0
rs1055345	21	*SLC19A1*	1797	0.29	0.10	0.6	1.0

Supplementary Table S4-6 (cont.) Corrected likelihood ratio test p-values of the interaction between LINE-1 methylation and SNPs in one-carbon metabolism pathway and bladder cancer susceptibility genes and in the SBS/EPICURO study

dbSNP (N=457)	Chromosome	Gene	N	MAF	*P* HWE	*P* LRT	Corrected *P* LRT
rs9980967	21	*SLC19A1*	1797	0.11	0.38	0.6	1.0
rs1870576	15	*MTHFS*	1780	0.46	0.06	0.6	1.0
rs473334	1	*CTH*	1793	0.31	0.81	0.6	1.0
rs663649	1	*CTH*	1793	0.31	0.81	0.6	1.0
rs6087990	20	*DNMT3B*	1797	0.32	0.64	0.6	1.0
rs11683778	2	*ATIC*	1795	0.03	0.54	0.6	1.0
rs6485991	11	*FOLH1*	1791	0.17	0.81	0.6	1.0
rs10839239	11	*FOLH1*	1795	0.23	0.85	0.6	1.0
rs12438477	15	*MTHFS*	1797	0.36	0.61	0.6	1.0
rs202700	11	*FOLH1*	1734	0.23	0.77	0.6	1.0
rs1465825	2	*DNMT3A*	1797	0.27	1.00	0.6	1.0
rs7175620	15	*MTHFS*	1797	0.22	0.14	0.6	1.0
rs16906158	11	*FOLH1*	1796	0.08	1.00	0.6	1.0
rs9306264	22	*TCN2*	1796	0.05	1.00	0.6	1.0
rs10460566	2	*DNMT3A*	1798	0.27	0.86	0.6	1.0
rs12482346	21	*SLC19A1*	1797	0.48	0.10	0.6	1.0
rs2790	18	*TYMS*	1789	0.20	0.67	0.6	1.0
rs2305230	3	*ALDH1L1*	1792	0.20	0.30	0.6	1.0
rs2236225	14	*MTHFD1*	1797	0.43	0.24	0.6	1.0
rs515064	1	*CTH*	1793	0.35	0.50	0.6	1.0
rs9977111	21	*SLC19A1*	1750	0.33	0.14	0.6	1.0
rs2304429	2	*DNMT3A*	1798	0.43	0.10	0.6	1.0
rs2066470	1	*MTHFR*	1788	0.08	0.39	0.6	1.0
rs202712	11	*FOLH1*	1796	0.23	0.85	0.6	1.0
rs2115536	15	*MTHFS*	1798	0.49	0.22	0.6	1.0
rs910085	20	*DNMT3B*	1797	0.29	0.93	0.6	1.0
rs17285431	15	*MTHFS*	1798	0.17	0.08	0.6	1.0
rs9323450	14	*MTHFD1*	1798	0.31	0.69	0.6	1.0
rs7177027	15	*MTHFS*	1797	0.24	0.40	0.6	1.0
rs3788190	21	*SLC19A1*	1795	0.47	0.10	0.6	1.0
rs1801198	22	*TCN2*	1797	0.43	0.08	0.6	1.0
rs11040353	11	*FOLH1*	1795	0.08	0.64	0.6	1.0
rs9305012	19	*DNMT1*	1797	0.08	0.64	0.6	1.0
rs10400277	11	*FOLH1*	1774	0.13	0.37	0.6	1.0
rs4911107	20	*DNMT3B*	1798	0.31	1.00	0.6	1.0
rs12222221	11	*FOLH1*	1798	0.08	1.00	0.6	1.0
rs11040390	11	*FOLH1*	1798	0.08	1.00	0.6	1.0
rs2372535	2	*ATIC*	1798	0.14	0.89	0.6	1.0
rs11040432	11	*FOLH1*	1797	0.08	1.00	0.6	1.0
NA	1	*MTR_01_ORDER*	1793	0.16	0.81	0.6	1.0

Supplementary Table S4-6 (cont.) Corrected likelihood ratio test p-values of the interaction between LINE-1 methylation and SNPs in one-carbon metabolism pathway and bladder cancer susceptibility genes and in the SBS/EPICURO study

dbSNP (N=457)	Chromosome	Gene	N	MAF	*P* HWE	*P* LRT	Corrected *P* LRT
rs8128681	21	*SLC19A1*	1798	0.33	0.94	0.6	1.0
rs2115540	15	*MTHFS*	1797	0.49	0.20	0.6	1.0
rs2035027	15	*MTHFS*	1798	0.16	0.80	0.7	1.0
rs234706	21	*CBS*	1793	0.33	0.36	0.7	1.0
rs600671	15	*MTHFS*	1797	0.45	0.34	0.7	1.0
rs9282690	3	*ALDH1L1*	1793	0.08	1.00	0.7	1.0
rs2162560	19	*DNMT1*	1797	0.38	0.94	0.7	1.0
rs2236224	14	*MTHFD1*	1798	0.36	0.07	0.7	1.0
rs1808119	2	*ATIC*	1797	0.19	0.82	0.7	1.0
rs6711622	2	*DNMT3A*	1798	0.44	1.00	0.7	1.0
rs9897362	17	*PEMT*	1798	0.06	0.77	0.7	1.0
rs12898642	15	*MTHFS*	1798	0.43	0.34	0.7	1.0
rs2287779	5	*MTRR*	1791	0.03	0.52	0.7	1.0
rs2287780	5	*MTRR*	1793	0.03	0.52	0.7	1.0
rs2586182	15	*MTHFS*	1798	0.14	0.78	0.7	1.0
rs2288349	19	*DNMT1*	1797	0.38	0.78	0.7	1.0
rs4778721	15	*MTHFS*	1798	0.22	0.09	0.7	1.0
rs4778719	15	*MTHFS*	1798	0.22	0.09	0.7	1.0
rs6511677	19	*DNMT1*	1797	0.38	0.89	0.7	1.0
rs282802	15	*MTHFS*	1798	0.29	0.09	0.7	1.0
rs16971450	15	*MTHFS*	1797	0.16	0.80	0.7	1.0
rs376863	15	*MTHFS*	1772	0.50	0.73	0.7	1.0
rs11687225	2	*ATIC*	1797	0.40	0.72	0.7	1.0
rs11158542	14	*MTHFD1*	1798	0.30	0.81	0.7	1.0
rs1369703	2	*DNMT3A*	1798	0.44	0.10	0.7	1.0
rs4646383	17	*PEMT*	1797	0.09	0.53	0.7	1.0
rs8101626	19	*DNMT1*	1798	0.39	0.89	0.7	1.0
rs898436	15	*MTHFS*	1793	0.45	0.37	0.7	1.0
rs11887120	2	*DNMT3A*	1798	0.41	0.44	0.7	1.0
rs10420338	19	*DNMT1*	1798	0.47	0.64	0.7	1.0
rs1805087	1	*MTR*	1793	0.16	0.81	0.7	1.0
rs1979276	17	*SHMT1*	1808	0.31	0.94	0.7	1.0
rs1888530	21	*SLC19A1*	1760	0.47	0.08	0.7	1.0
rs12121543	1	*MTHFR*	1793	0.22	0.32	0.7	1.0
rs11701960	21	*SLC19A1*	1797	0.18	0.91	0.7	1.0
rs8659	5	*MTRR*	1790	0.35	0.33	0.7	1.0
rs11158540	14	*MTHFD1*	1798	0.35	0.94	0.7	1.0
rs4779165	15	*MTHFS*	1797	0.16	0.80	0.7	1.0
rs11694842	2	*DNMT3A*	1797	0.28	0.36	0.7	1.0
rs7177659	15	*MTHFS*	1796	0.49	0.09	0.7	1.0

Supplementary Table S4-6 (cont.) Corrected likelihood ratio test p-values of the interaction between LINE-1 methylation and SNPs in one-carbon metabolism pathway and bladder cancer susceptibility genes and in the SBS/EPICURO study

dbSNP (N=457)	Chromosome	Gene	N	MAF	*P* HWE	*P* LRT	Corrected *P* LRT
rs1979277	17	*SHMT1*	1809	0.27	0.87	0.7	1.0
rs617219	5	*BHMT*	1790	0.32	0.94	0.7	1.0
rs13002567	2	*DNMT3A*	1798	0.28	0.40	0.7	1.0
rs7113075	11	*FOLH1*	1797	0.08	0.64	0.7	1.0
rs759920	19	*DNMT1*	1798	0.46	0.79	0.7	1.0
rs4673981	2	*ATIC*	1798	0.40	1.00	0.7	1.0
rs1081235	15	*MTHFS*	1798	0.20	0.67	0.7	1.0
rs17284990	15	*MTHFS*	1798	0.21	0.62	0.7	1.0
rs1667627	14	*MTHFD2*	1792	0.47	0.34	0.7	1.0
rs7085854	10	*AS3MT*	1797	0.22	0.85	0.7	1.0
rs7934591	11	*FOLH1*	1798	0.08	0.81	0.8	1.0
rs1473406	15	*MTHFS*	1796	0.15	0.79	0.8	1.0
rs11191457	10	*AS3MT*	1794	0.22	1.00	0.8	1.0
rs7594432	2	*DNMT3A*	1798	0.44	0.13	0.8	1.0
rs2228611	19	*DNMT1*	1798	0.46	0.79	0.8	1.0
rs3818239	14	*MTHFD1*	1787	0.13	0.66	0.8	1.0
rs282792	15	*MTHFS*	1798	0.37	0.47	0.8	1.0
rs1051298	21	*SLC19A1*	1790	0.47	0.17	0.8	1.0
rs12614943	2	*ATIC*	1798	0.27	0.73	0.8	1.0
NA	1	*MTR_01_2_i_order*	1793	0.16	0.81	0.8	1.0
rs2838977	21	*SLC19A1*	1796	0.40	0.72	0.8	1.0
rs1023159	21	*SLC19A1*	1796	0.42	0.78	0.8	1.0
rs4804122	19	*DNMT1*	1798	0.39	0.52	0.8	1.0
rs3772078	2	*ATIC*	1797	0.20	0.29	0.8	1.0
rs770144	15	*MTHFS*	1798	0.20	1.00	0.8	1.0
rs5749131	22	*TCN2*	1798	0.42	0.13	0.8	1.0
rs2877078	21	*SLC19A1*	1786	0.40	0.94	0.8	1.0
rs6058869	20	*DNMT3B*	1797	0.33	0.76	0.8	1.0
rs1164681	11	*FOLH1*	1798	0.12	0.25	0.8	1.0
rs1888533	21	*SLC19A1*	1797	0.48	0.42	0.8	1.0
rs2987981	14	*MTHFD1*	1798	0.26	1.00	0.8	1.0
rs9836592	3	*CHDH*	1798	0.32	0.14	0.8	1.0
rs1847638	11	*FOLH1*	1729	0.21	0.47	0.8	1.0
rs1256107	14	*MTHFD1*	1796	0.49	0.50	0.8	1.0
rs1051296	21	*SLC19A1*	1786	0.48	0.10	0.8	1.0
rs1846285	11	*FOLH1*	1795	0.16	0.54	0.8	1.0
rs35020344	14	*MTHFD1*	1797	0.48	0.42	0.8	1.0
rs6722613	2	*DNMT3A*	1798	0.40	0.89	0.8	1.0
rs12987326	2	*DNMT3A*	1798	0.37	0.47	0.8	1.0
rs11607791	11	*FOLH1*	1796	0.07	1.00	0.8	1.0

Supplementary Table S4-6 (cont.) Corrected likelihood ratio test p-values of the interaction between LINE-1 methylation and SNPs in one-carbon metabolism pathway and bladder cancer susceptibility genes and in the SBS/EPICURO study

dbSNP (N=457)	Chromosome	Gene	N	MAF	*P* HWE	*P* LRT	Corrected *P* LRT
rs7253062	19	*DNMT1*	1798	0.38	1.00	0.8	1.0
rs6445606	3	*CHDH*	1798	0.29	0.10	0.8	1.0
rs8041943	15	*MTHFS*	1794	0.41	0.37	0.8	1.0
rs2290684	19	*DNMT1*	1797	0.46	0.79	0.8	1.0
rs9642880	8	*MYC*	1797	0.44	0.78	0.8	1.0
rs1256114	14	*MTHFD1*	1797	0.11	0.86	0.8	1.0
rs8003379	14	*MTHFD1*	1796	0.25	0.78	0.8	1.0
rs6573559	14	*MTHFD1*	1798	0.30	0.94	0.8	1.0
rs2586154	15	*MTHFS*	1798	0.14	0.89	0.8	1.0
rs3821353	2	*ATIC*	1798	0.20	0.17	0.8	1.0
rs16853826	2	*ATIC*	1796	0.13	0.88	0.8	1.0
rs4987173	6	*GNMT*	1798	0.50	0.46	0.8	1.0
rs7929543	11	*FOLH1*	1798	0.07	0.81	0.8	1.0
rs8019804	14	*MTHFD1*	1798	0.07	1.00	0.8	1.0
rs11627387	14	*MTHFD1*	1798	0.30	0.81	0.8	1.0
rs1404774	2	*ATIC*	1784	0.22	0.42	0.8	1.0
rs16971249	15	*MTHFS*	1798	0.08	0.09	0.8	1.0
rs401681	5	*TERT-CLPTM1L*	1798	0.46	0.84	0.8	1.0
rs16853782	2	*ATIC*	1798	0.20	0.34	0.8	1.0
rs1801394	5	*MTRR*	1808	0.49	0.50	0.9	1.0
rs3740392	10	*AS3MT*	1794	0.29	0.41	0.9	1.0
rs2838970	21	*SLC19A1*	1797	0.40	0.78	0.9	1.0
rs2834231	21	*GART*	1798	0.26	0.73	0.9	1.0
rs10498034	2	*ATIC*	1798	0.16	0.90	0.9	1.0
rs282778	15	*MTHFS*	1798	0.25	0.86	0.9	1.0
rs16853834	2	*ATIC*	1798	0.17	0.71	0.9	1.0
rs2834232	21	*GART*	1797	0.26	0.73	0.9	1.0
rs8971	21	*GART*	1795	0.26	0.73	0.9	1.0
rs1256095	14	*MTHFD1*	1782	0.48	0.50	0.9	1.0
rs6058893	20	*DNMT3B*	1798	0.32	0.49	0.9	1.0
rs35709834	5	*DHFR*	1791	0.04	0.62	0.9	1.0
rs4779140	15	*MTHFS*	1797	0.48	0.79	0.9	1.0
rs8923	15	*MTHFS*	1798	0.08	0.15	0.9	1.0
rs2696935	11	*FOLH1*	1798	0.21	0.12	0.9	1.0
rs4441015	11	*FOLH1*	1753	0.14	0.40	0.9	1.0
rs2865908	11	*FOLH1*	1798	0.18	0.50	0.9	1.0
rs12373907	21	*SLC19A1*	1797	0.38	0.20	0.9	1.0
rs11085720	19	*DNMT1*	1798	0.41	0.44	0.9	1.0
rs34033751	11	*FOLH1*	1776	0.11	0.23	0.9	1.0
rs1256142	14	*MTHFD1*	1798	0.44	0.10	0.9	1.0

Supplementary Table S4-6 (cont.) Corrected likelihood ratio test p-values of the interaction between LINE-1 methylation and SNPs in one-carbon metabolism pathway and bladder cancer susceptibility genes and in the SBS/EPICURO study

dbSNP (N=457)	Chromosome	Gene	N	MAF	*P* HWE	*P* LRT	Corrected *P* LRT
rs767138	21	*SLC19A1*	1795	0.41	0.89	0.9	1.0
rs12613	21	*CBS*	1793	0.09	0.83	0.9	1.0
rs12999687	2	*DNMT3A*	1795	0.45	0.68	0.9	1.0
rs8128676	21	*SLC19A1*	1754	0.22	0.92	0.9	1.0
rs11658944	17	*PEMT*	1794	0.05	0.49	0.9	1.0
rs2983733	14	*MTHFD1*	1798	0.44	0.68	0.9	1.0
rs1046778	10	*AS3MT*	1798	0.32	0.70	0.9	1.0
rs4818789	21	*SLC19A1*	1798	0.25	0.06	0.9	1.0
rs17291414	19	*DNMT1*	1798	0.28	0.73	0.9	1.0
rs559062	1	*CTH*	1793	0.22	0.85	0.9	1.0
rs4094478	11	*FOLH1*	1770	0.20	0.34	0.9	1.0
rs35918857	19	*DNMT1*	1796	0.02	0.25	0.9	1.0
rs372447	15	*MTHFS*	1798	0.38	0.77	0.9	1.0
rs6087988	20	*DNMT3B*	1798	0.19	1.00	0.9	1.0
rs11677670	2	*DNMT3A*	1788	0.18	0.64	0.9	1.0
rs17279885	15	*MTHFS*	1798	0.20	0.67	0.9	1.0
rs11158538	14	*MTHFD1*	1795	0.45	0.78	0.9	1.0
rs17279753	15	*MTHFS*	1798	0.19	0.51	0.9	1.0
rs2696923	11	*FOLH1*	1798	0.21	0.12	0.9	1.0
rs2983736	14	*MTHFD1*	1793	0.44	0.73	0.9	1.0
rs13401241	2	*DNMT3A*	1798	0.45	0.28	0.9	1.0
rs567754	5	*BHMT*	1792	0.29	0.74	0.9	1.0
rs16999714	19	*DNMT1*	1796	0.21	0.26	0.9	1.0
rs11681447	2	*DNMT3A*	1796	0.29	0.62	0.9	1.0
rs2424932	20	*DNMT3B*	1797	0.43	0.15	0.9	1.0
rs8003567	14	*MTHFD1*	1798	0.11	0.47	0.9	1.0
rs6801605	3	*CHDH*	1798	0.37	1.00	0.9	1.0
rs8015278	14	*MTHFD1*	1798	0.07	0.18	0.9	1.0
rs1404772	2	*ATIC*	1798	0.08	0.34	1.0	1.0
rs685487	15	*MTHFS*	1798	0.36	0.34	1.0	1.0
rs2424908	20	*DNMT3B*	1798	0.17	0.40	1.0	1.0
rs1464864	2	*ATIC*	1798	0.30	0.81	1.0	1.0
rs734693	2	*DNMT3A*	1795	0.29	0.51	1.0	1.0
rs7587636	2	*DNMT3A*	1798	0.45	0.25	1.0	1.0
rs8068641	17	*PEMT*	1792	0.11	0.62	1.0	1.0
rs7144437	14	*MTHFD1*	1797	0.07	0.10	1.0	1.0
rs8011839	14	*MTHFD1*	1798	0.17	0.72	1.0	1.0
rs17751556	14	*MTHFD1*	1798	0.08	0.65	1.0	1.0
rs10769558	11	*FOLH1*	1797	0.21	0.18	1.0	1.0
rs10839210	11	*FOLH1*	1796	0.21	0.18	1.0	1.0

Supplementary Table S4-6 (cont.) Corrected likelihood ratio test p-values of the interaction between LINE-1 methylation and SNPs in one-carbon metabolism pathway and bladder cancer susceptibility genes and in the SBS/EPICURO study

dbSNP (N=457)	Chromosome	Gene	N	MAF	*P* HWE	*P* LRT	Corrected *P* LRT
rs2236222	14	*MTHFD1*	1798	0.10	0.27	1.0	1.0
rs2289093	2	*DNMT3A*	1798	0.29	0.62	1.0	1.0
rs11629135	14	*MTHFD1*	1797	0.11	0.38	1.0	1.0
rs12416687	10	*AS3MT*	1797	0.27	0.15	1.0	1.0
rs1076991	14	*MTHFD1*	1798	0.45	0.63	1.0	1.0
rs12148881	15	*MTHFS*	1797	0.26	0.19	1.0	1.0
rs4902278	14	*MTHFD1*	1791	0.07	0.18	1.0	1.0
rs749130	2	*DNMT3A*	1798	0.45	0.37	1.0	1.0
rs865646	5	*DHFR*	1736	0.36	0.55	1.0	1.0
rs12627639	21	*SLC19A1*	1797	0.21	0.68	1.0	1.0
rs8018032	14	*MTHFD1*	1798	0.45	0.95	1.0	1.0
rs663465	1	*CTH*	1792	0.42	0.36	1.0	1.0
rs2987969	14	*MTHFD1*	1797	0.45	0.95	1.0	1.0
rs2154583	21	*GART*	1791	0.40	0.67	1.0	1.0
rs6517178	21	*GART*	1798	0.40	0.72	1.0	1.0
rs11627525	14	*MTHFD1*	1798	0.11	0.08	1.0	1.0
rs2834235	21	*GART*	1797	0.39	0.67	1.0	1.0

Supplementary Table S4-6 (cont.) Corrected likelihood ratio test p-values of the interaction between LINE-1 methylation and SNPs in one-carbon metabolism pathway and bladder cancer susceptibility genes and in the SBS/EPICURO study

SNPs modeled in dominant mode of inheritance							
dbSNP (N=519)	Chromosome	Gene	N	MAF	*P* HWE	*P* LRT	Corrected *P* LRT
rs4646359	17	*PEMT*	1798	0.46	0.15	0.0007	0.3
rs11892429	2	*ATIC*	1798	0.29	1.00	0.0010	0.4
rs7604984	2	*ATIC*	1798	0.40	0.62	0.001	0.4
rs10197559	2	*ATIC*	1788	0.29	0.87	0.001	0.5
rs10498036	2	*ATIC*	1798	0.40	0.57	0.002	0.6
rs10179873	2	*ATIC*	1798	0.30	0.63	0.002	0.6
rs9890064	17	*PEMT*	1798	0.43	0.41	0.002	0.6
rs11855092	15	*MTHFS*	1798	0.24	0.31	0.002	0.7
rs10197653	2	*ATIC*	1798	0.29	0.74	0.003	0.8
rs1983462	2	*ATIC*	1798	0.31	0.47	0.003	0.8
rs4673993	2	*ATIC*	1798	0.32	0.31	0.004	0.9
rs4924922	17	*PEMT*	1798	0.37	0.43	0.004	0.9
rs6706415	2	*ATIC*	1798	0.31	0.81	0.004	0.9
rs4673991	2	*ATIC*	1797	0.32	0.31	0.004	0.9
rs4672768	2	*ATIC*	1794	0.32	0.31	0.005	0.9
rs4244599	17	*PEMT*	1774	0.47	0.34	0.009	1.0
rs435689	15	*MTHFS*	1798	0.49	0.50	0.009	1.0
rs11656215	17	*PEMT*	1798	0.46	0.20	0.010	1.0
rs12997662	2	*ATIC*	1798	0.34	0.26	0.02	1.0
rs2733106	15	*MTHFS*	1793	0.15	0.69	0.02	1.0
rs4646385	17	*PEMT*	1798	0.45	0.37	0.02	1.0
rs750546	17	*PEMT*	1773	0.45	0.19	0.02	1.0
rs12905663	15	*MTHFS*	1788	0.29	0.80	0.02	1.0
rs798766	4	*TMEM129-TACC3-FGFR3*	1798	0.18	0.21	0.02	1.0
rs6057645	20	*DNMT3B*	1797	0.04	0.22	0.03	1.0
rs4817580	21	*GART*	1797	0.10	0.70	0.03	1.0
rs2586153	15	*MTHFS*	1777	0.15	0.59	0.03	1.0
rs4673965	2	*ATIC*	1798	0.40	0.94	0.03	1.0
rs7581217	2	*DNMT3A*	1798	0.39	0.62	0.04	1.0
rs11683424	2	*DNMT3A*	1798	0.12	0.20	0.04	1.0
rs6749992	2	*DNMT3A*	1798	0.47	0.79	0.04	1.0
rs7604425	2	*ATIC*	1798	0.35	0.94	0.05	1.0
rs2289209	3	*CHDH*	1798	0.04	0.67	0.05	1.0
rs10165919	2	*ATIC*	1797	0.35	0.88	0.05	1.0
rs1880580	15	*MTHFS*	1798	0.31	0.38	0.06	1.0
rs2866358	11	*FOLH1*	1781	0.38	0.35	0.06	1.0
rs1074390	15	*MTHFS*	1798	0.38	0.77	0.06	1.0
rs1077965	15	*MTHFS*	1797	0.41	0.58	0.06	1.0
rs1917311	11	*FOLH1*	1753	0.40	0.72	0.06	1.0
rs7605753	2	*DNMT3A*	1797	0.47	0.68	0.06	1.0

Supplementary Table S4-6 (cont.) Corrected likelihood ratio test p-values of the interaction between LINE-1 methylation and SNPs in one-carbon metabolism pathway and bladder cancer susceptibility genes and in the SBS/EPICURO study

dbSNP (N=519)	Chromosome	Gene	N	MAF	*P* HWE	*P* LRT	Corrected *P* LRT
rs740234	22	*TCN2*	1798	0.23	0.39	0.07	1.0
rs7111215	11	*FOLH1*	1774	0.40	0.52	0.07	1.0
rs10509760	10	*AS3MT*	1798	0.13	0.88	0.07	1.0
rs3740394	10	*AS3MT*	1797	0.13	1.00	0.07	1.0
rs1164685	11	*FOLH1*	1793	0.38	0.47	0.08	1.0
rs4144700	11	*FOLH1*	1798	0.38	0.47	0.08	1.0
rs6119285	20	*DNMT3B*	1795	0.04	0.20	0.08	1.0
rs10839295	11	*FOLH1*	1797	0.40	0.48	0.08	1.0
rs1814175	11	*FOLH1*	1793	0.40	0.40	0.08	1.0
rs16988828	22	*TCN2*	1798	0.09	0.09	0.09	1.0
rs3862350	11	*FOLH1*	1765	0.40	0.15	0.09	1.0
rs1604503	15	*MTHFS*	1798	0.15	0.59	0.10	1.0
rs2733103	15	*MTHFS*	1797	0.15	0.51	0.10	1.0
rs11191439	10	*AS3MT*	1795	0.12	0.75	0.10	1.0
rs588458	11	*FOLH1*	1772	0.38	0.52	0.10	1.0
rs1380642	15	*MTHFS*	1798	0.18	0.57	0.1	1.0
rs282776	15	*MTHFS*	1798	0.36	0.47	0.1	1.0
rs748196	17	*PEMT*	1795	0.44	0.89	0.1	1.0
rs914238	21	*SLC19A1*	1798	0.49	0.89	0.1	1.0
rs9835128	3	*CHDH*	1797	0.16	0.38	0.1	1.0
rs4819130	21	*SLC19A1*	1794	0.45	0.15	0.1	1.0
rs1051266	21	*SLC19A1*	1798	0.45	0.20	0.1	1.0
rs4393531	15	*MTHFS*	1795	0.47	0.63	0.1	1.0
rs282787	15	*MTHFS*	1798	0.04	0.34	0.1	1.0
rs4610054	2	*ATIC*	1794	0.38	0.67	0.1	1.0
rs11040416	11	*FOLH1*	1798	0.42	0.58	0.1	1.0
rs11040421	11	*FOLH1*	1798	0.14	0.78	0.2	1.0
rs2183601	21	*SLC19A1*	1797	0.20	0.92	0.2	1.0
rs2150460	21	*SLC19A1*	1798	0.20	1.00	0.2	1.0
rs6760069	2	*ATIC*	1797	0.15	0.35	0.2	1.0
rs3177999	21	*SLC19A1*	1790	0.46	0.17	0.2	1.0
rs10380	5	*MTRR*	1793	0.10	0.56	0.2	1.0
rs10932605	2	*ATIC*	1798	0.14	0.39	0.2	1.0
rs4434082	21	*SLC19A1*	1797	0.20	1.00	0.2	1.0
rs3788200	21	*SLC19A1*	1798	0.45	0.31	0.2	1.0
rs9606756	22	*TCN2*	1798	0.12	0.87	0.2	1.0
rs2330183	21	*SLC19A1*	1757	0.45	0.05	0.2	1.0
rs2241807	3	*CHDH*	1798	0.42	0.89	0.2	1.0
rs282814	15	*MTHFS*	1798	0.22	0.84	0.2	1.0
rs3862342	11	*FOLH1*	1795	0.28	0.93	0.2	1.0

Supplementary Table S4-6 (cont.) Corrected likelihood ratio test p-values of the interaction between LINE-1 methylation and SNPs in one-carbon metabolism pathway and bladder cancer susceptibility genes and in the SBS/EPICURO study

dbSNP (N=519)	Chromosome	Gene	N	MAF	*P* HWE	*P* LRT	Corrected *P* LRT
rs914232	21	*SLC19A1*	1797	0.45	0.17	0.2	1.0
rs4369857	2	*ATIC*	1798	0.04	1.00	0.2	1.0
rs1950902	14	*MTHFD1*	1798	0.15	1.00	0.2	1.0
NA	1	*MTHFR_02_ORDER*	1658	0.39	0.33	0.2	1.0
rs6518253	21	*SLC19A1*	1797	0.46	0.63	0.2	1.0
rs17211644	15	*MTHFS*	1798	0.10	0.72	0.2	1.0
rs2838973	21	*SLC19A1*	1798	0.20	0.92	0.2	1.0
rs4819128	21	*SLC19A1*	1798	0.45	0.13	0.2	1.0
rs914231	21	*SLC19A1*	1791	0.45	0.19	0.2	1.0
rs443394	15	*MTHFS*	1798	0.42	0.30	0.2	1.0
rs10839296	11	*FOLH1*	1777	0.25	0.65	0.2	1.0
rs9980967	21	*SLC19A1*	1797	0.11	0.38	0.2	1.0
rs9976878	21	*SLC19A1*	1797	0.20	1.00	0.2	1.0
rs34048824	2	*DNMT3A*	1797	0.51	0.74	0.2	1.0
rs7107178	11	*FOLH1*	1796	0.25	0.86	0.2	1.0
rs4779148	15	*MTHFS*	1798	0.10	0.45	0.2	1.0
rs9306139	21	*SLC19A1*	1796	0.20	1.00	0.2	1.0
rs1127717	3	*ALDH1L1*	1793	0.24	1.00	0.2	1.0
rs7111711	11	*FOLH1*	1798	0.25	0.86	0.2	1.0
rs2294008	8	*PSCA*	1798	0.46	0.73	0.2	1.0
rs7124266	11	*FOLH1*	1798	0.30	0.94	0.2	1.0
rs4646344	17	*PEMT*	1798	0.46	0.45	0.2	1.0
rs7583409	2	*DNMT3A*	1795	0.36	0.38	0.2	1.0
rs10501325	11	*FOLH1*	1798	0.06	0.08	0.2	1.0
rs663649	1	*CTH*	1793	0.31	0.81	0.2	1.0
rs473334	1	*CTH*	1793	0.31	0.81	0.2	1.0
rs12995968	2	*DNMT3A*	1798	0.04	0.66	0.2	1.0
rs6058897	20	*DNMT3B*	1798	0.44	0.17	0.2	1.0
rs12482346	21	*SLC19A1*	1797	0.48	0.10	0.2	1.0
rs1550117	2	*DNMT3A*	1797	0.08	0.81	0.2	1.0
rs7102702	11	*FOLH1*	1741	0.04	1.00	0.2	1.0
rs1917321	11	*FOLH1*	1790	0.50	0.54	0.3	1.0
rs7117247	11	*FOLH1*	1798	0.06	0.08	0.3	1.0
rs9462856	6	*GNMT*	1798	0.42	0.53	0.3	1.0
rs6087990	20	*DNMT3B*	1797	0.32	0.64	0.3	1.0
rs2116940	19	*DNMT1*	1797	0.08	0.64	0.3	1.0
rs5749135	22	*TCN2*	1798	0.43	0.07	0.3	1.0
rs12797843	11	*FOLH1*	1798	0.13	0.37	0.3	1.0
rs3788190	21	*SLC19A1*	1795	0.47	0.10	0.3	1.0
rs4911108	20	*DNMT3B*	1793	0.28	0.87	0.3	1.0

Supplementary Table S4-6 (cont.) Corrected likelihood ratio test p-values of the interaction between LINE-1 methylation and SNPs in one-carbon metabolism pathway and bladder cancer susceptibility genes and in the SBS/EPICURO study

dbSNP (N=519)	Chromosome	Gene	N	MAF	*P* HWE	*P* LRT	Corrected *P* LRT
rs6757920	2	*ATIC*	1794	0.48	0.09	0.3	1.0
rs3760188	17	*PEMT*	1798	0.46	0.38	0.3	1.0
rs17824591	14	*MTHFD1*	1796	0.23	0.63	0.3	1.0
rs1870576	15	*MTHFS*	1780	0.46	0.06	0.3	1.0
rs6495441	15	*MTHFS*	1798	0.25	0.93	0.3	1.0
rs1802059	5	*MTRR*	1792	0.36	0.60	0.3	1.0
rs2424913	20	*DNMT3B*	1797	0.37	0.39	0.3	1.0
rs11040387	11	*FOLH1*	1785	0.07	0.31	0.3	1.0
rs4532960	10	*AS3MT*	1797	0.44	0.89	0.3	1.0
rs1650697	5	*DHFR*	1791	0.23	0.06	0.3	1.0
rs2301955	22	*TCN2*	1794	0.43	0.10	0.3	1.0
rs10748835	10	*AS3MT*	1798	0.44	0.89	0.3	1.0
rs1801131	1	*MTHFR*	1710	0.28	0.34	0.3	1.0
rs6485991	11	*FOLH1*	1791	0.17	0.81	0.3	1.0
rs7276295	21	*SLC19A1*	1798	0.06	0.56	0.3	1.0
rs2838964	21	*SLC19A1*	1798	0.06	0.77	0.3	1.0
rs17285431	15	*MTHFS*	1798	0.17	0.08	0.3	1.0
rs202700	11	*FOLH1*	1734	0.23	0.77	0.3	1.0
rs7085104	10	*AS3MT*	1798	0.38	1.00	0.3	1.0
rs282772	15	*MTHFS*	1798	0.14	0.25	0.3	1.0
rs2162560	19	*DNMT1*	1797	0.38	0.94	0.3	1.0
rs17209637	15	*MTHFS*	1794	0.26	0.79	0.3	1.0
rs515064	1	*CTH*	1793	0.35	0.50	0.3	1.0
rs1369703	2	*DNMT3A*	1798	0.44	0.10	0.3	1.0
rs12462004	19	*DNMT1*	1795	0.08	0.64	0.3	1.0
rs4911107	20	*DNMT3B*	1798	0.31	1.00	0.3	1.0
rs2035027	15	*MTHFS*	1798	0.16	0.80	0.3	1.0
rs660439	11	*FOLH1*	1794	0.23	0.85	0.3	1.0
rs11869600	17	*PEMT*	1796	0.37	0.38	0.3	1.0
rs910085	20	*DNMT3B*	1797	0.29	0.93	0.3	1.0
rs2838965	21	*SLC19A1*	1792	0.42	0.33	0.3	1.0
rs3783	17	*SHMT1*	1806	0.26	0.30	0.3	1.0
rs376863	15	*MTHFS*	1772	0.50	0.73	0.3	1.0
rs3785499	17	*PEMT*	1798	0.48	0.54	0.3	1.0
rs2283873	22	*TCN2*	1779	0.03	0.49	0.3	1.0
rs6511677	19	*DNMT1*	1797	0.38	0.89	0.3	1.0
rs2838958	21	*SLC19A1*	1794	0.46	0.63	0.3	1.0
rs4476347	2	*ATIC*	1798	0.25	0.28	0.3	1.0
rs16906158	11	*FOLH1*	1796	0.08	1.00	0.3	1.0
rs12797853	11	*FOLH1*	1794	0.13	0.46	0.3	1.0

Supplementary Table S4-6 (cont.) Corrected likelihood ratio test p-values of the interaction between LINE-1 methylation and SNPs in one-carbon metabolism pathway and bladder cancer susceptibility genes and in the SBS/EPICURO study

dbSNP (N=519)	Chromosome	Gene	N	MAF	*P* HWE	*P* LRT	Corrected *P* LRT
rs7563206	2	*ATIC*	1796	0.47	0.84	0.3	1.0
rs10839239	11	*FOLH1*	1795	0.23	0.85	0.3	1.0
rs7219568	17	*PEMT*	1798	0.06	0.52	0.3	1.0
rs16971450	15	*MTHFS*	1797	0.16	0.80	0.3	1.0
rs4804125	19	*DNMT1*	1797	0.08	0.64	0.3	1.0
rs202712	11	*FOLH1*	1796	0.23	0.85	0.4	1.0
rs2288350	19	*DNMT1*	1798	0.08	0.64	0.4	1.0
rs10420338	19	*DNMT1*	1798	0.47	0.64	0.4	1.0
rs4531931	2	*ATIC*	1796	0.31	0.23	0.4	1.0
rs4817579	21	*GART*	1798	0.34	0.15	0.4	1.0
rs853858	20	*DNMT3B*	1796	0.37	0.19	0.4	1.0
rs8111085	19	*DNMT1*	1797	0.08	0.64	0.4	1.0
rs6141813	20	*DNMT3B*	1798	0.14	1.00	0.4	1.0
rs4817577	21	*GART*	1797	0.34	0.20	0.4	1.0
rs600671	15	*MTHFS*	1797	0.45	0.34	0.4	1.0
rs7283354	21	*GART*	1798	0.34	0.15	0.4	1.0
rs9977111	21	*SLC19A1*	1750	0.33	0.14	0.4	1.0
rs10948059	6	*GNMT*	1778	0.49	0.54	0.4	1.0
rs11672909	19	*DNMT1*	1797	0.08	0.81	0.4	1.0
rs4925048	17	*PEMT*	1797	0.10	0.17	0.4	1.0
rs8034036	15	*MTHFS*	1795	0.11	0.23	0.4	1.0
rs6058896	20	*DNMT3B*	1797	0.09	0.67	0.4	1.0
rs17279286	15	*MTHFS*	1797	0.05	0.73	0.4	1.0
rs2288349	19	*DNMT1*	1797	0.38	0.78	0.4	1.0
rs6058894	20	*DNMT3B*	1797	0.08	1.00	0.4	1.0
rs4646364	17	*PEMT*	1797	0.03	0.15	0.4	1.0
rs2424906	20	*DNMT3B*	1798	0.37	0.28	0.4	1.0
rs8112895	19	*DNMT1*	1798	0.08	0.81	0.4	1.0
rs759920	19	*DNMT1*	1798	0.46	0.79	0.4	1.0
rs12121543	1	*MTHFR*	1793	0.22	0.32	0.4	1.0
rs1014971	22	*CBX6 APOBEC3A*	1797	0.30	0.23	0.4	1.0
rs7220132	17	*PEMT*	1798	0.29	0.57	0.4	1.0
rs4779165	15	*MTHFS*	1797	0.16	0.80	0.4	1.0
rs9789571	2	*ATIC*	1798	0.42	0.89	0.4	1.0
rs582172	15	*MTHFS*	1798	0.42	0.45	0.4	1.0
rs8101626	19	*DNMT1*	1798	0.39	0.89	0.4	1.0
rs2305230	3	*ALDH1L1*	1792	0.20	0.30	0.4	1.0
rs898436	15	*MTHFS*	1793	0.45	0.37	0.4	1.0
rs10418707	19	*DNMT1*	1796	0.08	0.64	0.4	1.0
rs4479310	17	*PEMT*	1798	0.30	0.63	0.4	1.0

Supplementary Table S4-6 (cont.) Corrected likelihood ratio test p-values of the interaction between LINE-1 methylation and SNPs in one-carbon metabolism pathway and bladder cancer susceptibility genes and in the SBS/EPICURO study

dbSNP (N=519)	Chromosome	Gene	N	MAF	*P* HWE	*P* LRT	Corrected *P* LRT
rs11871738	17	*PEMT*	1798	0.38	0.67	0.4	1.0
rs9001	3	*CHDH*	1793	0.09	0.21	0.4	1.0
rs4819138	21	*SLC19A1*	1797	0.40	0.89	0.4	1.0
rs9332	5	*MTRR*	1793	0.12	0.34	0.4	1.0
rs4804494	19	*DNMT1*	1797	0.08	0.64	0.4	1.0
rs12591436	15	*MTHFS*	1798	0.35	0.55	0.4	1.0
rs13002567	2	*DNMT3A*	1798	0.28	0.40	0.4	1.0
rs7594432	2	*DNMT3A*	1798	0.44	0.13	0.4	1.0
rs4804490	19	*DNMT1*	1796	0.08	0.64	0.4	1.0
rs1808119	2	*ATIC*	1797	0.19	0.82	0.4	1.0
rs2228611	19	*DNMT1*	1798	0.46	0.79	0.4	1.0
rs1081235	15	*MTHFS*	1798	0.20	0.67	0.4	1.0
rs1880586	2	*ATIC*	1797	0.47	0.89	0.4	1.0
rs7560488	2	*DNMT3A*	1731	0.48	0.15	0.4	1.0
rs7951180	11	*FOLH1*	1778	0.17	0.55	0.4	1.0
rs12438477	15	*MTHFS*	1797	0.36	0.61	0.4	1.0
rs1956545	14	*MTHFD1*	1797	0.08	0.17	0.4	1.0
rs10400277	11	*FOLH1*	1774	0.13	0.37	0.4	1.0
NA	1	*MTHFR_02_2_i_order*	1793	0.39	0.35	0.4	1.0
rs1801133	1	*MTHFR*	1793	0.39	0.35	0.4	1.0
rs2424914	20	*DNMT3B*	1798	0.39	0.39	0.4	1.0
rs6058883	20	*DNMT3B*	1797	0.39	0.36	0.4	1.0
rs1109859	17	*PEMT*	1770	0.18	0.64	0.4	1.0
rs4673981	2	*ATIC*	1798	0.40	1.00	0.4	1.0
rs1888530	21	*SLC19A1*	1760	0.47	0.08	0.4	1.0
rs11887120	2	*DNMT3A*	1798	0.41	0.44	0.4	1.0
rs9282690	3	*ALDH1L1*	1793	0.08	1.00	0.4	1.0
rs11040106	11	*FOLH1*	1787	0.36	0.66	0.4	1.0
NA	1	*MTR_01_ORDER*	1793	0.16	0.81	0.4	1.0
rs648372	11	*FOLH1*	1772	0.16	0.46	0.4	1.0
rs1051298	21	*SLC19A1*	1790	0.47	0.17	0.4	1.0
rs2834233	21	*GART*	1797	0.09	0.08	0.4	1.0
rs8102137	19	*CCNE1*	1798	0.33	0.13	0.4	1.0
rs2790	18	*TYMS*	1789	0.20	0.67	0.4	1.0
rs2236224	14	*MTHFD1*	1798	0.36	0.07	0.4	1.0
rs10839229	11	*FOLH1*	1795	0.08	0.35	0.4	1.0
rs709046	20	*DNMT3B*	1798	0.03	1.00	0.5	1.0
rs4804122	19	*DNMT1*	1798	0.39	0.52	0.5	1.0
rs4646404	17	*PEMT*	1792	0.35	0.26	0.5	1.0
rs1051296	21	*SLC19A1*	1786	0.48	0.10	0.5	1.0

Supplementary Table S4-6 (cont.) Corrected likelihood ratio test p-values of the interaction between LINE-1 methylation and SNPs in one-carbon metabolism pathway and bladder cancer susceptibility genes and in the SBS/EPICURO study

dbSNP (N=519)	Chromosome	Gene	N	MAF	*P* HWE	*P* LRT	Corrected *P* LRT
rs234706	21	*CBS*	1793	0.33	0.36	0.5	1.0
rs2424921	20	*DNMT3B*	1798	0.39	0.32	0.5	1.0
rs3960835	11	*FOLH1*	1798	0.06	0.12	0.5	1.0
rs5753231	22	*TCN2*	1797	0.16	0.62	0.5	1.0
rs2115536	15	*MTHFS*	1798	0.49	0.22	0.5	1.0
rs1055345	21	*SLC19A1*	1797	0.29	0.10	0.5	1.0
rs6058869	20	*DNMT3B*	1797	0.33	0.76	0.5	1.0
rs1809986	11	*FOLH1*	1798	0.36	0.38	0.5	1.0
rs11694842	2	*DNMT3A*	1797	0.28	0.36	0.5	1.0
rs2267163	22	*TCN2*	1794	0.43	0.06	0.5	1.0
rs202676	11	*FOLH1*	1788	0.17	0.72	0.5	1.0
rs1460177	15	*MTHFS*	1797	0.08	0.37	0.5	1.0
rs2290684	19	*DNMT1*	1797	0.46	0.79	0.5	1.0
rs16971253	15	*MTHFS*	1797	0.10	1.00	0.5	1.0
rs16971252	15	*MTHFS*	1798	0.06	0.34	0.5	1.0
rs897453	17	*PEMT*	1790	0.47	0.54	0.5	1.0
rs679470	11	*FOLH1*	1797	0.17	0.63	0.5	1.0
rs202718	11	*FOLH1*	1798	0.15	0.79	0.5	1.0
rs16971231	15	*MTHFS*	1794	0.05	0.14	0.5	1.0
rs10839224	11	*FOLH1*	1798	0.08	0.35	0.5	1.0
rs11683778	2	*ATIC*	1796	0.03	0.54	0.5	1.0
rs2877078	21	*SLC19A1*	1786	0.40	0.94	0.5	1.0
rs4495895	11	*FOLH1*	1751	0.09	0.39	0.5	1.0
rs2424928	20	*DNMT3B*	1798	0.39	0.26	0.5	1.0
rs7929543	11	*FOLH1*	1798	0.07	0.81	0.5	1.0
rs6058891	20	*DNMT3B*	1796	0.39	0.32	0.5	1.0
rs11701960	21	*SLC19A1*	1797	0.18	0.91	0.5	1.0
rs17556442	11	*FOLH1*	1792	0.06	0.12	0.5	1.0
rs445263	15	*MTHFS*	1798	0.29	0.68	0.5	1.0
rs11892031	2	*UGT1A*	1798	0.09	1.00	0.5	1.0
rs9621049	22	*TCN2*	1798	0.11	0.86	0.5	1.0
rs11158540	14	*MTHFD1*	1798	0.35	0.94	0.5	1.0
rs9305012	19	*DNMT1*	1797	0.08	0.64	0.5	1.0
rs7113251	11	*FOLH1*	1798	0.06	0.12	0.5	1.0
rs5749131	22	*TCN2*	1798	0.42	0.13	0.5	1.0
rs2586182	15	*MTHFS*	1798	0.14	0.78	0.5	1.0
rs3755817	3	*CHDH*	1797	0.30	0.42	0.5	1.0
rs2838977	21	*SLC19A1*	1796	0.40	0.72	0.5	1.0
rs6119286	20	*DNMT3B*	1798	0.02	0.38	0.5	1.0
rs7217764	17	*PEMT*	1798	0.05	0.51	0.5	1.0

Supplementary Table S4-6 (cont.) Corrected likelihood ratio test p-values of the interaction between LINE-1 methylation and SNPs in one-carbon metabolism pathway and bladder cancer susceptibility genes and in the SBS/EPICURO study

dbSNP (N=519)	Chromosome	Gene	N	MAF	*P* HWE	*P* LRT	Corrected *P* LRT
rs1473406	15	*MTHFS*	1796	0.15	0.79	0.5	1.0
rs1846285	11	*FOLH1*	1795	0.16	0.54	0.5	1.0
rs7120743	11	*FOLH1*	1774	0.36	0.77	0.5	1.0
NA	1	*MTR_01_2_i_order*	1793	0.16	0.81	0.5	1.0
rs1805087	1	*MTR*	1793	0.16	0.81	0.5	1.0
rs282778	15	*MTHFS*	1798	0.25	0.86	0.5	1.0
rs2304429	2	*DNMT3A*	1798	0.43	0.10	0.5	1.0
rs632220	11	*FOLH1*	1798	0.09	0.30	0.5	1.0
rs8003379	14	*MTHFD1*	1796	0.25	0.78	0.5	1.0
rs1801198	22	*TCN2*	1797	0.43	0.08	0.5	1.0
rs16853834	2	*ATIC*	1798	0.17	0.71	0.5	1.0
rs282802	15	*MTHFS*	1798	0.29	0.09	0.6	1.0
rs9974061	21	*SLC19A1*	1798	0.18	0.82	0.6	1.0
rs17728676	11	*FOLH1*	1797	0.06	0.14	0.6	1.0
rs4779140	15	*MTHFS*	1797	0.48	0.79	0.6	1.0
rs4987173	6	*GNMT*	1798	0.50	0.46	0.6	1.0
rs12913164	15	*MTHFS*	1798	0.08	0.34	0.6	1.0
rs11607791	11	*FOLH1*	1796	0.07	1.00	0.6	1.0
rs3897953	15	*MTHFS*	1798	0.10	0.25	0.6	1.0
rs710521	3	*TP63*	1798	0.26	0.93	0.6	1.0
rs2424922	20	*DNMT3B*	1796	0.39	0.29	0.6	1.0
rs7085854	10	*AS3MT*	1797	0.22	0.85	0.6	1.0
rs683680	11	*FOLH1*	1797	0.09	0.14	0.6	1.0
rs770144	15	*MTHFS*	1798	0.20	1.00	0.6	1.0
rs12592743	15	*MTHFS*	1798	0.10	0.12	0.6	1.0
rs1888533	21	*SLC19A1*	1797	0.48	0.42	0.6	1.0
rs11191457	10	*AS3MT*	1794	0.22	1.00	0.6	1.0
rs17745484	2	*DNMT3A*	1797	0.35	0.30	0.6	1.0
rs9323450	14	*MTHFD1*	1798	0.31	0.69	0.6	1.0
rs6750194	2	*ATIC*	1784	0.07	0.62	0.6	1.0
rs3818239	14	*MTHFD1*	1787	0.13	0.66	0.6	1.0
rs10498514	14	*MTHFD1*	1798	0.03	0.56	0.6	1.0
rs7253062	19	*DNMT1*	1798	0.38	1.00	0.6	1.0
rs2115540	15	*MTHFS*	1797	0.49	0.20	0.6	1.0
rs9306264	22	*TCN2*	1797	0.05	1.00	0.6	1.0
rs650826	11	*FOLH1*	1796	0.09	0.14	0.6	1.0
rs2275566	1	*MTR*	1793	0.41	0.53	0.6	1.0
rs4779141	15	*MTHFS*	1793	0.34	0.55	0.6	1.0
rs1495741	8	*MYC*	1798	0.24	1.00	0.6	1.0
rs1256112	14	*MTHFD1*	1798	0.40	0.29	0.6	1.0

Supplementary Table S4-6 (cont.) Corrected likelihood ratio test p-values of the interaction between LINE-1 methylation and SNPs in one-carbon metabolism pathway and bladder cancer susceptibility genes and in the SBS/EPICURO study

dbSNP (N=519)	Chromosome	Gene	N	MAF	*P* HWE	*P* LRT	Corrected *P* LRT
rs4820887	22	*TCN2*	1798	0.10	0.85	0.6	1.0
rs8074191	17	*PEMT*	1778	0.28	0.93	0.6	1.0
rs8659	5	*MTRR*	1790	0.35	0.33	0.6	1.0
rs1023159	21	*SLC19A1*	1796	0.42	0.78	0.6	1.0
rs598841	11	*FOLH1*	1791	0.08	0.19	0.6	1.0
rs13036246	2	*DNMT3A*	1797	0.48	0.08	0.6	1.0
rs11892646	2	*DNMT3A*	1797	0.11	0.87	0.6	1.0
rs7358352	11	*FOLH1*	1797	0.09	0.30	0.6	1.0
rs10418	22	*TCN2*	1772	0.21	0.41	0.6	1.0
rs8074074	17	*PEMT*	1796	0.30	0.87	0.6	1.0
rs12098986	11	*FOLH1*	1795	0.09	0.20	0.6	1.0
rs6058893	20	*DNMT3B*	1798	0.32	0.49	0.6	1.0
rs7117025	11	*FOLH1*	1798	0.09	0.30	0.6	1.0
rs16853826	2	*ATIC*	1796	0.13	0.88	0.6	1.0
rs11040263	11	*FOLH1*	1795	0.09	0.19	0.6	1.0
rs12910340	15	*MTHFS*	1798	0.42	0.73	0.6	1.0
rs12293923	11	*FOLH1*	1792	0.09	0.19	0.6	1.0
rs8128681	21	*SLC19A1*	1798	0.33	0.94	0.6	1.0
rs12373907	21	*SLC19A1*	1797	0.38	0.20	0.6	1.0
rs11687225	2	*ATIC*	1797	0.40	0.72	0.6	1.0
rs3893384	15	*MTHFS*	1798	0.42	0.44	0.6	1.0
rs2287779	5	*MTRR*	1791	0.03	0.52	0.6	1.0
rs1256107	14	*MTHFD1*	1796	0.49	0.50	0.6	1.0
rs2287780	5	*MTRR*	1793	0.03	0.52	0.6	1.0
rs1081231	15	*MTHFS*	1797	0.17	0.71	0.6	1.0
rs1256095	14	*MTHFD1*	1782	0.48	0.50	0.7	1.0
rs2586154	15	*MTHFS*	1798	0.14	0.89	0.7	1.0
rs7586294	2	*DNMT3A*	1797	0.47	0.73	0.7	1.0
rs8128676	21	*SLC19A1*	1754	0.22	0.92	0.7	1.0
rs4646410	17	*PEMT*	1795	0.31	0.94	0.7	1.0
rs1979277	17	*SHMT1*	1809	0.27	0.87	0.7	1.0
rs2983733	14	*MTHFD1*	1798	0.44	0.68	0.7	1.0
rs378057	15	*MTHFS*	1797	0.14	0.89	0.7	1.0
rs4911263	20	*DNMT3B*	1798	0.32	1.00	0.7	1.0
rs6087988	20	*DNMT3B*	1798	0.19	1.00	0.7	1.0
rs12999687	2	*DNMT3A*	1795	0.45	0.68	0.7	1.0
rs7279305	21	*SLC19A1*	1797	0.35	1.00	0.7	1.0
rs6413471	1	*CTH*	1793	0.05	0.72	0.7	1.0
rs13427202	2	*DNMT3A*	1797	0.47	0.68	0.7	1.0
rs11677670	2	*DNMT3A*	1788	0.18	0.64	0.7	1.0

Supplementary Table S4-6 (cont.) Corrected likelihood ratio test p-values of the interaction between LINE-1 methylation and SNPs in one-carbon metabolism pathway and bladder cancer susceptibility genes and in the SBS/EPICURO study

dbSNP (N=519)	Chromosome	Gene	N	MAF	*P* HWE	*P* LRT	Corrected *P* LRT
rs1819409	11	*FOLH1*	1783	0.09	0.10	0.7	1.0
rs7926211	11	*FOLH1*	1794	0.09	0.19	0.7	1.0
rs920253	3	*CHDH*	1798	0.02	0.08	0.7	1.0
rs1164681	11	*FOLH1*	1798	0.12	0.25	0.7	1.0
rs12898670	15	*MTHFS*	1796	0.34	0.70	0.7	1.0
rs2066470	1	*MTHFR*	1788	0.08	0.39	0.7	1.0
rs10163099	15	*MTHFS*	1792	0.26	0.48	0.7	1.0
rs12900076	15	*MTHFS*	1793	0.08	0.25	0.7	1.0
rs1131603	22	*TCN2*	1797	0.04	0.66	0.7	1.0
rs12987326	2	*DNMT3A*	1798	0.37	0.47	0.7	1.0
rs10498034	2	*ATIC*	1798	0.16	0.90	0.7	1.0
rs11040261	11	*FOLH1*	1797	0.09	0.39	0.7	1.0
rs2295639	14	*MTHFD1*	1797	0.03	0.54	0.7	1.0
rs35020344	14	*MTHFD1*	1797	0.48	0.42	0.7	1.0
rs9836592	3	*CHDH*	1798	0.32	0.14	0.7	1.0
rs1404772	2	*ATIC*	1798	0.08	0.34	0.7	1.0
rs6722613	2	*DNMT3A*	1798	0.40	0.89	0.7	1.0
rs7175620	15	*MTHFS*	1797	0.22	0.14	0.7	1.0
rs12614943	2	*ATIC*	1798	0.27	0.73	0.7	1.0
rs1020697	17	*PEMT*	1770	0.10	0.35	0.7	1.0
rs2838970	21	*SLC19A1*	1797	0.40	0.78	0.7	1.0
rs2983736	14	*MTHFD1*	1793	0.44	0.73	0.7	1.0
rs6495446	15	*MTHFS*	1797	0.26	0.38	0.7	1.0
rs17284990	15	*MTHFS*	1798	0.21	0.62	0.7	1.0
rs9897362	17	*PEMT*	1798	0.06	0.77	0.7	1.0
rs767138	21	*SLC19A1*	1795	0.41	0.89	0.7	1.0
rs8068641	17	*PEMT*	1792	0.11	0.62	0.7	1.0
rs17101854	14	*MTHFD1*	1797	0.03	0.53	0.7	1.0
rs4818789	21	*SLC19A1*	1798	0.25	0.06	0.7	1.0
rs437302	20	*DNMT3B*	1797	0.08	1.00	0.7	1.0
rs16971260	15	*MTHFS*	1798	0.04	1.00	0.7	1.0
rs1979276	17	*SHMT1*	1808	0.31	0.94	0.7	1.0
rs1046778	10	*AS3MT*	1798	0.32	0.70	0.7	1.0
rs13401241	2	*DNMT3A*	1798	0.45	0.28	0.7	1.0
rs166868	15	*MTHFS*	1796	0.37	0.94	0.7	1.0
rs7946	17	*PEMT*	1798	0.30	0.52	0.7	1.0
rs8081810	17	*PEMT*	1797	0.20	0.92	0.7	1.0
rs16971249	15	*MTHFS*	1798	0.08	0.09	0.8	1.0
rs4778721	15	*MTHFS*	1798	0.22	0.09	0.8	1.0
rs4778719	15	*MTHFS*	1798	0.22	0.09	0.8	1.0

Supplementary Table S4-6 (cont.) Corrected likelihood ratio test p-values of the interaction between LINE-1 methylation and SNPs in one-carbon metabolism pathway and bladder cancer susceptibility genes and in the SBS/EPICURO study

dbSNP (N=519)	Chromosome	Gene	N	MAF	*P* HWE	*P* LRT	Corrected *P* LRT
rs1464864	2	*ATIC*	1798	0.30	0.81	0.8	1.0
rs3772078	2	*ATIC*	1797	0.20	0.29	0.8	1.0
rs8015278	14	*MTHFD1*	1798	0.07	0.18	0.8	1.0
rs16853782	2	*ATIC*	1798	0.20	0.34	0.8	1.0
rs7587636	2	*DNMT3A*	1798	0.45	0.25	0.8	1.0
rs7113075	11	*FOLH1*	1797	0.08	0.64	0.8	1.0
rs2424908	20	*DNMT3B*	1798	0.17	0.40	0.8	1.0
rs7177027	15	*MTHFS*	1797	0.24	0.40	0.8	1.0
rs617219	5	*BHMT*	1790	0.32	0.94	0.8	1.0
rs4441015	11	*FOLH1*	1753	0.14	0.40	0.8	1.0
rs7177659	15	*MTHFS*	1796	0.49	0.09	0.8	1.0
rs2838961	21	*SLC19A1*	1797	0.34	0.94	0.8	1.0
rs6586282	21	*CBS*	1792	0.18	0.16	0.8	1.0
rs12903985	15	*MTHFS*	1797	0.29	0.14	0.8	1.0
rs1667627	14	*MTHFD2*	1792	0.47	0.34	0.8	1.0
rs17279885	15	*MTHFS*	1798	0.20	0.67	0.8	1.0
rs12148881	15	*MTHFS*	1797	0.26	0.19	0.8	1.0
rs7144437	14	*MTHFD1*	1797	0.07	0.10	0.8	1.0
rs2275565	1	*MTR*	1793	0.19	0.66	0.8	1.0
rs2281603	14	*MTHFD1*	1798	0.20	0.34	0.8	1.0
rs2372535	2	*ATIC*	1798	0.14	0.89	0.8	1.0
rs2236225	14	*MTHFD1*	1797	0.43	0.24	0.8	1.0
rs7934591	11	*FOLH1*	1798	0.08	0.81	0.8	1.0
rs3821353	2	*ATIC*	1798	0.20	0.17	0.8	1.0
rs12482067	21	*GART*	1798	0.02	0.24	0.8	1.0
rs7575625	2	*DNMT3A*	1797	0.47	0.89	0.8	1.0
rs6713377	2	*DNMT3A*	1797	0.47	0.89	0.8	1.0
rs4646383	17	*PEMT*	1797	0.09	0.53	0.8	1.0
rs3740392	10	*AS3MT*	1794	0.29	0.41	0.8	1.0
rs1256142	14	*MTHFD1*	1798	0.44	0.10	0.8	1.0
rs8041943	15	*MTHFS*	1794	0.41	0.37	0.8	1.0
rs2865908	11	*FOLH1*	1798	0.18	0.50	0.8	1.0
rs2424932	20	*DNMT3B*	1797	0.43	0.15	0.8	1.0
rs1076991	14	*MTHFD1*	1798	0.45	0.63	0.8	1.0
rs8129445	21	*SLC19A1*	1798	0.32	0.70	0.8	1.0
rs34033751	11	*FOLH1*	1776	0.11	0.23	0.8	1.0
rs16999714	19	*DNMT1*	1796	0.21	0.26	0.8	1.0
rs944422	21	*SLC19A1*	1791	0.35	0.76	0.8	1.0
rs3774616	3	*CHDH*	1798	0.04	1.00	0.8	1.0
rs11040353	11	*FOLH1*	1795	0.08	0.64	0.8	1.0

Supplementary Table S4-6 (cont.) Corrected likelihood ratio test p-values of the interaction between LINE-1 methylation and SNPs in one-carbon metabolism pathway and bladder cancer susceptibility genes and in the SBS/EPICURO study

dbSNP (N=519)	Chromosome	Gene	N	MAF	*P* HWE	*P* LRT	Corrected *P* LRT
rs35709834	5	*DHFR*	1793	0.04	0.62	0.8	1.0
rs17291414	19	*DNMT1*	1798	0.28	0.73	0.8	1.0
rs1801394	5	*MTRR*	1808	0.49	0.50	0.8	1.0
rs559062	1	*CTH*	1793	0.22	0.85	0.8	1.0
rs8923	15	*MTHFS*	1798	0.08	0.15	0.9	1.0
rs12222221	11	*FOLH1*	1798	0.08	1.00	0.9	1.0
rs11040390	11	*FOLH1*	1798	0.08	1.00	0.9	1.0
rs1404774	2	*ATIC*	1784	0.22	0.42	0.9	1.0
rs749130	2	*DNMT3A*	1798	0.45	0.37	0.9	1.0
rs4902278	14	*MTHFD1*	1791	0.07	0.18	0.9	1.0
rs11158538	14	*MTHFD1*	1795	0.45	0.78	0.9	1.0
rs734693	2	*DNMT3A*	1795	0.29	0.51	0.9	1.0
rs17279753	15	*MTHFS*	1798	0.19	0.51	0.9	1.0
rs9642880	8	*MYC*	1797	0.44	0.78	0.9	1.0
rs12627639	21	*SLC19A1*	1797	0.21	0.68	0.9	1.0
rs12613	21	*CBS*	1793	0.09	0.83	0.9	1.0
rs6711622	2	*DNMT3A*	1798	0.44	1.00	0.9	1.0
rs2987981	14	*MTHFD1*	1798	0.26	1.00	0.9	1.0
rs11040432	11	*FOLH1*	1797	0.08	1.00	0.9	1.0
rs8011839	14	*MTHFD1*	1798	0.17	0.72	0.9	1.0
rs865646	5	*DHFR*	1736	0.36	0.55	0.9	1.0
rs7102641	11	*FOLH1*	1793	0.08	0.47	0.9	1.0
rs372447	15	*MTHFS*	1798	0.38	0.77	0.9	1.0
rs12884767	14	*MTHFD1*	1798	0.04	0.14	0.9	1.0
rs9910747	17	*PEMT*	1798	0.07	0.47	0.9	1.0
rs8128050	21	*SLC19A1*	1794	0.34	0.94	0.9	1.0
rs567754	5	*BHMT*	1792	0.29	0.74	0.9	1.0
rs8129350	21	*SLC19A1*	1797	0.34	0.76	0.9	1.0
rs1059394	18	*TYMS*	1791	0.31	0.64	0.9	1.0
rs7759302	6	*GNMT*	1798	0.06	0.57	0.9	1.0
rs699517	18	*TYMS*	1791	0.31	0.64	0.9	1.0
rs663465	1	*CTH*	1792	0.42	0.36	0.9	1.0
rs4094478	11	*FOLH1*	1770	0.20	0.34	0.9	1.0
rs11085720	19	*DNMT1*	1798	0.41	0.44	0.9	1.0
rs10839210	11	*FOLH1*	1796	0.21	0.18	0.9	1.0
rs13317328	3	*CHDH*	1798	0.09	0.06	0.9	1.0
rs8003567	14	*MTHFD1*	1798	0.11	0.47	0.9	1.0
rs6445606	3	*CHDH*	1798	0.29	0.10	0.9	1.0
rs2289093	2	*DNMT3A*	1798	0.29	0.62	0.9	1.0
rs685487	15	*MTHFS*	1798	0.36	0.34	0.9	1.0

Supplementary Table S4-6 (cont.) Corrected likelihood ratio test p-values of the interaction between LINE-1 methylation and SNPs in one-carbon metabolism pathway and bladder cancer susceptibility genes and in the SBS/EPICURO study

dbSNP (N=519)	Chromosome	Gene	N	MAF	*P* HWE	*P* LRT	Corrected *P* LRT
rs1256114	14	*MTHFD1*	1797	0.11	0.86	0.9	1.0
rs2987969	14	*MTHFD1*	1797	0.45	0.95	0.9	1.0
rs11627387	14	*MTHFD1*	1798	0.30	0.81	0.9	1.0
rs35918857	19	*DNMT1*	1797	0.02	0.25	0.9	1.0
rs1465825	2	*DNMT3A*	1797	0.27	1.00	0.9	1.0
rs8018032	14	*MTHFD1*	1798	0.45	0.95	0.9	1.0
rs282795	15	*MTHFS*	1797	0.32	0.88	0.9	1.0
rs3783728	14	*MTHFD1*	1798	0.08	0.64	0.9	1.0
rs1847638	11	*FOLH1*	1729	0.21	0.47	0.9	1.0
rs2696923	11	*FOLH1*	1798	0.21	0.12	0.9	1.0
rs12416687	10	*AS3MT*	1797	0.27	0.15	0.9	1.0
rs17751556	14	*MTHFD1*	1798	0.08	0.65	1.0	1.0
rs10769558	11	*FOLH1*	1797	0.21	0.18	1.0	1.0
rs6517178	21	*GART*	1798	0.40	0.72	1.0	1.0
rs11681447	2	*DNMT3A*	1796	0.29	0.62	1.0	1.0
rs2236222	14	*MTHFD1*	1798	0.10	0.27	1.0	1.0
rs12898642	15	*MTHFS*	1798	0.43	0.34	1.0	1.0
rs6801605	3	*CHDH*	1798	0.37	1.00	1.0	1.0
rs2696935	11	*FOLH1*	1798	0.21	0.12	1.0	1.0
rs401681	5	*TERT-CLPTM1L*	1798	0.46	0.84	1.0	1.0
rs3800292	6	*GNMT*	1798	0.06	0.57	1.0	1.0
rs11852515	15	*MTHFS*	1798	0.11	0.74	1.0	1.0
rs8019804	14	*MTHFD1*	1798	0.07	1.00	1.0	1.0
rs11158542	14	*MTHFD1*	1798	0.30	0.81	1.0	1.0
rs2834235	21	*GART*	1797	0.39	0.67	1.0	1.0
rs282792	15	*MTHFS*	1798	0.37	0.47	1.0	1.0
rs11627525	14	*MTHFD1*	1798	0.11	0.08	1.0	1.0
rs10460566	2	*DNMT3A*	1798	0.27	0.86	1.0	1.0
rs12453139	17	*PEMT*	1797	0.26	0.66	1.0	1.0
rs7174668	15	*MTHFS*	1798	0.21	0.48	1.0	1.0
rs11658944	17	*PEMT*	1797	0.05	0.49	1.0	1.0
rs2154583	21	*GART*	1791	0.40	0.67	1.0	1.0
rs11629135	14	*MTHFD1*	1797	0.11	0.38	1.0	1.0
rs8971	21	*GART*	1795	0.26	0.73	1.0	1.0
rs2834232	21	*GART*	1797	0.26	0.73	1.0	1.0
rs2834231	21	*GART*	1798	0.26	0.73	1.0	1.0
rs204942	15	*MTHFS*	1798	0.21	0.31	1.0	1.0
rs6573559	14	*MTHFD1*	1798	0.30	0.94	1.0	1.0
rs2838969	21	*SLC19A1*	1798	0.07	0.79	1.0	1.0

Supplementary Table S4-6 (cont.) Corrected likelihood ratio test p-values of the interaction between LINE-1 methylation and SNPs in one-carbon metabolism pathway and bladder cancer susceptibility genes and in the SBS/EPICURO study

SNPs modeled in recessive mode of inheritance							
dbSNP (N=446)	Chromosome	Gene	N	MAF	*P* HWE	*P* LRT	Corrected *P* LRT
rs7560488	2	*DNMT3A*	1731	0.48	0.15	0.003	0.8
rs282814	15	*MTHFS*	1798	0.22	0.84	0.005	0.9
rs11683424	2	*DNMT3A*	1798	0.12	0.20	0.005	0.9
rs6495441	15	*MTHFS*	1798	0.25	0.93	0.006	0.9
rs204942	15	*MTHFS*	1798	0.21	0.31	0.007	1.0
rs4646404	17	*PEMT*	1792	0.35	0.26	0.009	1.0
rs4925048	17	*PEMT*	1797	0.10	0.17	0.01	1.0
rs7174668	15	*MTHFS*	1798	0.21	0.48	0.01	1.0
rs12903985	15	*MTHFS*	1797	0.29	0.14	0.01	1.0
rs7563206	2	*ATIC*	1796	0.47	0.84	0.01	1.0
rs11869600	17	*PEMT*	1796	0.37	0.38	0.02	1.0
rs11871738	17	*PEMT*	1798	0.38	0.67	0.02	1.0
rs1077965	15	*MTHFS*	1797	0.41	0.58	0.02	1.0
rs1880580	15	*MTHFS*	1798	0.31	0.38	0.02	1.0
rs1880586	2	*ATIC*	1797	0.47	0.89	0.02	1.0
rs7581217	2	*DNMT3A*	1798	0.39	0.62	0.02	1.0
rs798766	4	*TMEM129-TACC3-FGFR3*	1798	0.18	0.21	0.02	1.0
rs282795	15	*MTHFS*	1797	0.32	0.88	0.02	1.0
rs4646344	17	*PEMT*	1798	0.46	0.45	0.03	1.0
rs11852515	15	*MTHFS*	1798	0.11	0.74	0.04	1.0
rs3760188	17	*PEMT*	1798	0.46	0.38	0.04	1.0
rs10418	22	*TCN2*	1772	0.21	0.41	0.04	1.0
rs12898670	15	*MTHFS*	1796	0.34	0.70	0.05	1.0
rs6713377	2	*DNMT3A*	1797	0.47	0.89	0.05	1.0
rs17209637	15	*MTHFS*	1794	0.26	0.79	0.05	1.0
rs11892646	2	*DNMT3A*	1797	0.11	0.87	0.05	1.0
rs1495741	8	*NAT2*	1798	0.24	1.00	0.05	1.0
rs7575625	2	*DNMT3A*	1797	0.47	0.89	0.06	1.0
rs17824591	14	*MTHFD1*	1796	0.23	0.63	0.06	1.0
rs944422	21	*SLC19A1*	1791	0.35	0.76	0.06	1.0
rs8129350	21	*SLC19A1*	1797	0.34	0.76	0.07	1.0
rs1014971	22	*CBXB APOBEC3A*	1797	0.30	0.23	0.07	1.0
rs2838961	21	*SLC19A1*	1797	0.34	0.94	0.08	1.0
rs166868	15	*MTHFS*	1796	0.37	0.94	0.09	1.0
rs1460177	15	*MTHFS*	1797	0.08	0.37	0.09	1.0
rs16971253	15	*MTHFS*	1797	0.10	1.00	0.09	1.0
rs9001	3	*CHDH*	1793	0.09	0.21	0.10	1.0
rs7586294	2	*DNMT3A*	1797	0.47	0.73	0.1	1.0
rs10380	5	*MTRR*	1793	0.10	0.56	0.1	1.0
rs4531931	2	*ATIC*	1796	0.31	0.23	0.1	1.0

Supplementary Table S4-6 (cont.) Corrected likelihood ratio test p-values of the interaction between LINE-1 methylation and SNPs in one-carbon metabolism pathway and bladder cancer susceptibility genes and in the SBS/EPICURO study

dbSNP (N=446)	Chromosome	Gene	N	MAF	*P* HWE	*P* LRT	Corrected *P* LRT
rs8128050	21	*SLC19A1*	1794	0.34	0.94	0.1	1.0
rs13427202	2	*DNMT3A*	1797	0.47	0.68	0.1	1.0
rs710521	3	*TP63*	1798	0.26	0.93	0.1	1.0
rs6706415	2	*ATIC*	1798	0.31	0.81	0.1	1.0
rs1983462	2	*ATIC*	1798	0.31	0.47	0.1	1.0
rs435689	15	*MTHFS*	1798	0.49	0.50	0.1	1.0
rs4476347	2	*ATIC*	1798	0.25	0.28	0.1	1.0
rs13317328	3	*CHDH*	1798	0.09	0.06	0.1	1.0
rs7279305	21	*SLC19A1*	1797	0.35	1.00	0.1	1.0
rs8102137	19	*CCNE1*	1798	0.33	0.13	0.1	1.0
rs582172	15	*MTHFS*	1798	0.42	0.45	0.1	1.0
rs6586282	21	*CBS*	1792	0.18	0.16	0.1	1.0
rs10197653	2	*ATIC*	1798	0.29	0.74	0.1	1.0
rs6058897	20	*DNMT3B*	1798	0.44	0.17	0.2	1.0
rs13036246	2	*DNMT3A*	1797	0.48	0.08	0.2	1.0
rs10179873	2	*ATIC*	1798	0.30	0.63	0.2	1.0
rs11656215	17	*PEMT*	1798	0.46	0.20	0.2	1.0
rs897453	17	*PEMT*	1790	0.47	0.54	0.2	1.0
rs2424913	20	*DNMT3B*	1797	0.37	0.39	0.2	1.0
rs1059394	18	*TYMS*	1791	0.31	0.64	0.2	1.0
rs9332	5	*MTRR*	1793	0.12	0.34	0.2	1.0
rs7583409	2	*DNMT3A*	1795	0.36	0.38	0.2	1.0
rs2275565	1	*MTR*	1793	0.19	0.66	0.2	1.0
rs1081231	15	*MTHFS*	1797	0.17	0.71	0.2	1.0
rs7220132	17	*PEMT*	1798	0.29	0.57	0.2	1.0
rs2424906	20	*DNMT3B*	1798	0.37	0.28	0.2	1.0
rs699517	18	*TYMS*	1791	0.31	0.64	0.2	1.0
rs10163099	15	*MTHFS*	1792	0.26	0.48	0.2	1.0
rs8129445	21	*SLC19A1*	1798	0.32	0.70	0.2	1.0
rs378057	15	*MTHFS*	1797	0.14	0.89	0.2	1.0
rs4779141	15	*MTHFS*	1793	0.34	0.55	0.2	1.0
rs12910340	15	*MTHFS*	1798	0.42	0.73	0.2	1.0
rs4646410	17	*PEMT*	1795	0.31	0.94	0.2	1.0
rs1109859	17	*PEMT*	1770	0.18	0.64	0.2	1.0
rs12453139	17	*PEMT*	1797	0.26	0.66	0.2	1.0
rs6058891	20	*DNMT3B*	1796	0.39	0.32	0.2	1.0
rs10498036	2	*ATIC*	1798	0.40	0.57	0.2	1.0
rs8074074	17	*PEMT*	1796	0.30	0.87	0.2	1.0
rs9835128	3	*CHDH*	1797	0.16	0.38	0.2	1.0
rs6495446	15	*MTHFS*	1797	0.26	0.38	0.2	1.0

Supplementary Table S4-6 (cont.) Corrected likelihood ratio test p-values of the interaction between LINE-1 methylation and SNPs in one-carbon metabolism pathway and bladder cancer susceptibility genes and in the SBS/EPICURO study

dbSNP (N=446)	Chromosome	Gene	N	MAF	*P* HWE	*P* LRT	Corrected *P* LRT
rs1802059	5	*MTRR*	1792	0.36	0.60	0.2	1.0
rs4393531	15	*MTHFS*	1795	0.47	0.63	0.2	1.0
rs8034036	15	*MTHFS*	1795	0.11	0.23	0.2	1.0
rs2838965	21	*SLC19A1*	1792	0.42	0.33	0.3	1.0
rs1256112	14	*MTHFD1*	1798	0.40	0.29	0.3	1.0
rs445263	15	*MTHFS*	1798	0.29	0.68	0.3	1.0
rs7175620	15	*MTHFS*	1797	0.22	0.14	0.3	1.0
rs2275566	1	*MTR*	1793	0.41	0.53	0.3	1.0
rs2424922	20	*DNMT3B*	1796	0.39	0.29	0.3	1.0
rs9606756	22	*TCN2*	1798	0.12	0.87	0.3	1.0
rs5753231	22	*TCN2*	1797	0.16	0.62	0.3	1.0
rs4673991	2	*ATIC*	1797	0.32	0.31	0.3	1.0
rs4672768	2	*ATIC*	1794	0.32	0.31	0.3	1.0
rs2281603	14	*MTHFD1*	1798	0.20	0.34	0.3	1.0
rs9897362	17	*PEMT*	1798	0.06	0.77	0.3	1.0
rs4673993	2	*ATIC*	1798	0.32	0.31	0.3	1.0
rs2424928	20	*DNMT3B*	1798	0.39	0.26	0.3	1.0
rs11040353	11	*FOLH1*	1795	0.08	0.64	0.3	1.0
rs6058883	20	*DNMT3B*	1797	0.39	0.36	0.3	1.0
rs7604984	2	*ATIC*	1798	0.40	0.62	0.3	1.0
rs7946	17	*PEMT*	1798	0.30	0.52	0.3	1.0
rs11040432	11	*FOLH1*	1797	0.08	1.00	0.3	1.0
rs12222221	11	*FOLH1*	1798	0.08	1.00	0.3	1.0
rs11040390	11	*FOLH1*	1798	0.08	1.00	0.3	1.0
rs2424914	20	*DNMT3B*	1798	0.39	0.39	0.3	1.0
rs8081810	17	*PEMT*	1797	0.20	0.92	0.3	1.0
rs7177027	15	*MTHFS*	1797	0.24	0.40	0.3	1.0
rs11892429	2	*ATIC*	1798	0.29	1.00	0.3	1.0
rs2372535	2	*ATIC*	1798	0.14	0.89	0.3	1.0
rs2424921	20	*DNMT3B*	1798	0.39	0.32	0.3	1.0
rs4817577	21	*GART*	1797	0.34	0.20	0.3	1.0
rs11040421	11	*FOLH1*	1798	0.14	0.78	0.3	1.0
rs1465825	2	*DNMT3A*	1797	0.27	1.00	0.3	1.0
rs10460566	2	*DNMT3A*	1798	0.27	0.86	0.3	1.0
rs1055345	21	*SLC19A1*	1797	0.29	0.10	0.3	1.0
rs4244599	17	*PEMT*	1774	0.47	0.34	0.3	1.0
rs2838973	21	*SLC19A1*	1798	0.20	0.92	0.3	1.0
rs853858	20	*DNMT3B*	1796	0.37	0.19	0.3	1.0
rs6711622	2	*DNMT3A*	1798	0.44	1.00	0.3	1.0
rs3893384	15	*MTHFS*	1798	0.42	0.44	0.3	1.0

Supplementary Table S4-6 (cont.) Corrected likelihood ratio test p-values of the interaction between LINE-1 methylation and SNPs in one-carbon metabolism pathway and bladder cancer susceptibility genes and in the SBS/EPICURO study

dbSNP (N=446)	Chromosome	Gene	N	MAF	*P* HWE	*P* LRT	Corrected *P* LRT
rs10948059	6	*GNMT*	1778	0.49	0.54	0.3	1.0
rs7283354	21	*GART*	1798	0.34	0.15	0.3	1.0
rs10197559	2	*ATIC*	1788	0.29	0.87	0.3	1.0
rs2236225	14	*MTHFD1*	1797	0.43	0.24	0.3	1.0
rs1127717	3	*ALDH1L1*	1793	0.24	1.00	0.3	1.0
rs2115540	15	*MTHFS*	1797	0.49	0.20	0.3	1.0
rs3755817	3	*CHDH*	1797	0.30	0.42	0.3	1.0
rs10165919	2	*ATIC*	1797	0.35	0.88	0.3	1.0
rs2267163	22	*TCN2*	1794	0.43	0.06	0.3	1.0
rs7604425	2	*ATIC*	1798	0.35	0.94	0.3	1.0
rs6757920	2	*ATIC*	1794	0.48	0.09	0.3	1.0
rs8074191	17	*PEMT*	1778	0.28	0.93	0.3	1.0
rs914238	21	*SLC19A1*	1798	0.49	0.89	0.3	1.0
rs2115536	15	*MTHFS*	1798	0.49	0.22	0.3	1.0
rs3862350	11	*FOLH1*	1765	0.40	0.15	0.4	1.0
rs11158542	14	*MTHFD1*	1798	0.30	0.81	0.4	1.0
rs4817579	21	*GART*	1798	0.34	0.15	0.4	1.0
rs4911263	20	*DNMT3B*	1798	0.32	1.00	0.4	1.0
rs12997662	2	*ATIC*	1798	0.34	0.26	0.4	1.0
rs5749135	22	*TCN2*	1798	0.43	0.07	0.4	1.0
rs17745484	2	*DNMT3A*	1797	0.35	0.30	0.4	1.0
rs4646350	17	*PEMT*	1798	0.36	0.83	0.4	1.0
rs1814175	11	*FOLH1*	1793	0.40	0.40	0.4	1.0
rs4778721	15	*MTHFS*	1798	0.22	0.09	0.4	1.0
rs4778719	15	*MTHFS*	1798	0.22	0.09	0.4	1.0
rs10839295	11	*FOLH1*	1797	0.40	0.48	0.4	1.0
rs1801198	22	*TCN2*	1797	0.43	0.08	0.4	1.0
rs9789571	2	*ATIC*	1798	0.42	0.89	0.4	1.0
rs12898642	15	*MTHFS*	1798	0.43	0.34	0.4	1.0
rs1380642	15	*MTHFS*	1798	0.18	0.57	0.4	1.0
rs1667627	14	*MTHFD2*	1792	0.47	0.34	0.4	1.0
rs4646383	17	*PEMT*	1797	0.09	0.53	0.4	1.0
rs6749992	2	*DNMT3A*	1798	0.47	0.79	0.4	1.0
rs7605753	2	*DNMT3A*	1797	0.47	0.68	0.4	1.0
rs6518253	21	*SLC19A1*	1797	0.46	0.63	0.4	1.0
rs2124344	17	*PEMT*	1797	0.36	0.77	0.4	1.0
rs1809986	11	*FOLH1*	1798	0.36	0.38	0.4	1.0
rs2183601	21	*SLC19A1*	1797	0.20	0.92	0.4	1.0
rs282792	15	*MTHFS*	1798	0.37	0.47	0.4	1.0
rs2304429	2	*DNMT3A*	1798	0.43	0.10	0.4	1.0

Supplementary Table S4-6 (cont.) Corrected likelihood ratio test p-values of the interaction between LINE-1 methylation and SNPs in one-carbon metabolism pathway and bladder cancer susceptibility genes and in the SBS/EPICURO study

dbSNP (N=446)	Chromosome	Gene	N	MAF	*P* HWE	*P* LRT	Corrected *P* LRT
rs9974061	21	*SLC19A1*	1798	0.18	0.82	0.4	1.0
rs7085104	10	*AS3MT*	1798	0.38	1.00	0.4	1.0
rs7934591	11	*FOLH1*	1798	0.08	0.81	0.4	1.0
NA	1	*MTHFR*	1658	0.39	0.33	0.4	1.0
rs7113075	11	*FOLH1*	1797	0.08	0.64	0.4	1.0
rs9462856	6	*GNMT*	1798	0.42	0.53	0.4	1.0
NA	1	*MTHFR_02_2_i_order*	1793	0.39	0.35	0.4	1.0
rs1801133	1	*MTHFR*	1793	0.39	0.35	0.4	1.0
rs748196	17	*PEMT*	1795	0.44	0.89	0.4	1.0
rs2301955	22	*TCN2*	1794	0.43	0.10	0.4	1.0
rs2066470	1	*MTHFR*	1788	0.08	0.39	0.4	1.0
rs2305230	3	*ALDH1L1*	1792	0.20	0.30	0.4	1.0
rs3785499	17	*PEMT*	1798	0.48	0.54	0.4	1.0
rs4610054	2	*ATIC*	1794	0.38	0.67	0.4	1.0
rs7120743	11	*FOLH1*	1774	0.36	0.77	0.4	1.0
rs3862342	11	*FOLH1*	1795	0.28	0.93	0.4	1.0
rs4646385	17	*PEMT*	1798	0.45	0.37	0.4	1.0
rs2294008	8	*PSCA*	1798	0.46	0.73	0.5	1.0
rs9976878	21	*SLC19A1*	1797	0.20	1.00	0.5	1.0
rs4646359	17	*PEMT*	1798	0.46	0.15	0.5	1.0
rs4479310	17	*PEMT*	1798	0.30	0.63	0.5	1.0
rs1979276	17	*SHMT1*	1808	0.31	0.94	0.5	1.0
rs9306139	21	*SLC19A1*	1796	0.20	1.00	0.5	1.0
rs7951180	11	*FOLH1*	1778	0.17	0.55	0.5	1.0
rs12797853	11	*FOLH1*	1794	0.13	0.46	0.5	1.0
rs7177659	15	*MTHFS*	1796	0.49	0.09	0.5	1.0
rs1956545	14	*MTHFD1*	1797	0.08	0.17	0.5	1.0
rs1164681	11	*FOLH1*	1798	0.12	0.25	0.5	1.0
rs1256114	14	*MTHFD1*	1797	0.11	0.86	0.5	1.0
rs282776	15	*MTHFS*	1798	0.36	0.47	0.5	1.0
rs11627387	14	*MTHFD1*	1798	0.30	0.81	0.5	1.0
rs6573559	14	*MTHFD1*	1798	0.30	0.94	0.5	1.0
rs1950902	14	*MTHFD1*	1798	0.15	1.00	0.5	1.0
NA	1	*MTR*	1793	0.16	0.81	0.5	1.0
rs9642880	8	*MYC*	1797	0.44	0.78	0.5	1.0
rs740234	22	*TCN2*	1798	0.23	0.39	0.5	1.0
rs9323450	14	*MTHFD1*	1798	0.31	0.69	0.5	1.0
rs1847638	11	*FOLH1*	1729	0.21	0.47	0.5	1.0
rs2150460	21	*SLC19A1*	1798	0.20	1.00	0.5	1.0
rs282772	15	*MTHFS*	1798	0.14	0.25	0.5	1.0

Supplementary Table S4-6 (cont.) Corrected likelihood ratio test p-values of the interaction between LINE-1 methylation and SNPs in one-carbon metabolism pathway and bladder cancer susceptibility genes and in the SBS/EPICURO study

dbSNP (N=446)	Chromosome	Gene	N	MAF	*P* HWE	*P* LRT	Corrected *P* LRT
rs3740394	10	*AS3MT*	1797	0.13	1.00	0.5	1.0
rs11887120	2	*DNMT3A*	1798	0.41	0.44	0.5	1.0
rs10509760	10	*AS3MT*	1798	0.13	0.88	0.5	1.0
rs1805087	1	*MTR*	1793	0.16	0.81	0.5	1.0
rs4924922	17	*PEMT*	1798	0.37	0.43	0.5	1.0
rs34048824	2	*DNMT3A*	1797	0.51	0.74	0.5	1.0
rs401681	5	*TERT-CLPTM1L*	1798	0.46	0.84	0.5	1.0
rs17284990	15	*MTHFS*	1798	0.21	0.62	0.5	1.0
rs2834231	21	*GART*	1798	0.26	0.73	0.5	1.0
rs2834232	21	*GART*	1797	0.26	0.73	0.5	1.0
rs8971	21	*GART*	1795	0.26	0.73	0.5	1.0
rs8019804	14	*MTHFD1*	1798	0.07	1.00	0.5	1.0
rs4819138	21	*SLC19A1*	1797	0.40	0.89	0.5	1.0
rs679470	11	*FOLH1*	1797	0.17	0.63	0.5	1.0
rs7107178	11	*FOLH1*	1796	0.25	0.86	0.5	1.0
rs4532960	10	*AS3MT*	1797	0.44	0.89	0.6	1.0
rs7111711	11	*FOLH1*	1798	0.25	0.86	0.6	1.0
rs4817580	21	*GART*	1797	0.10	0.70	0.6	1.0
rs6445606	3	*CHDH*	1798	0.29	0.10	0.6	1.0
rs10748835	10	*AS3MT*	1798	0.44	0.89	0.6	1.0
rs3772078	2	*ATIC*	1797	0.20	0.29	0.6	1.0
rs10839296	11	*FOLH1*	1777	0.25	0.65	0.6	1.0
rs4434082	21	*SLC19A1*	1797	0.20	1.00	0.6	1.0
rs35020344	14	*MTHFD1*	1797	0.48	0.42	0.6	1.0
rs3783	17	*SHMT1*	1806	0.26	0.30	0.6	1.0
rs648372	11	*FOLH1*	1772	0.16	0.46	0.6	1.0
rs770144	15	*MTHFS*	1798	0.20	1.00	0.6	1.0
rs8659	5	*MTRR*	1790	0.35	0.33	0.6	1.0
rs11040106	11	*FOLH1*	1787	0.36	0.66	0.6	1.0
rs443394	15	*MTHFS*	1798	0.42	0.30	0.6	1.0
rs17211644	15	*MTHFS*	1798	0.10	0.72	0.6	1.0
rs1801394	5	*MTRR*	1808	0.49	0.50	0.6	1.0
rs34033751	11	*FOLH1*	1776	0.11	0.23	0.6	1.0
rs6141813	20	*DNMT3B*	1798	0.14	1.00	0.6	1.0
rs3821353	2	*ATIC*	1798	0.20	0.17	0.6	1.0
rs202718	11	*FOLH1*	1798	0.15	0.79	0.6	1.0
rs8128681	21	*SLC19A1*	1798	0.33	0.94	0.6	1.0
rs1604503	15	*MTHFS*	1798	0.15	0.59	0.6	1.0
rs2834233	21	*GART*	1797	0.09	0.08	0.6	1.0
rs202676	11	*FOLH1*	1788	0.17	0.72	0.6	1.0

Supplementary Table S4-6 (cont.) Corrected likelihood ratio test p-values of the interaction between LINE-1 methylation and SNPs in one-carbon metabolism pathway and bladder cancer susceptibility genes and in the SBS/EPICURO study

dbSNP (N=446)	Chromosome	Gene	N	MAF	*P* HWE	*P* LRT	Corrected *P* LRT
rs910085	20	*DNMT3B*	1797	0.29	0.93	0.6	1.0
rs10400277	11	*FOLH1*	1774	0.13	0.37	0.6	1.0
rs4911108	20	*DNMT3B*	1793	0.28	0.87	0.6	1.0
rs2987981	14	*MTHFD1*	1798	0.26	1.00	0.6	1.0
rs1256107	14	*MTHFD1*	1796	0.49	0.50	0.6	1.0
rs11687225	2	*ATIC*	1797	0.40	0.72	0.6	1.0
rs2838970	21	*SLC19A1*	1797	0.40	0.78	0.6	1.0
rs9836592	3	*CHDH*	1798	0.32	0.14	0.6	1.0
rs4441015	11	*FOLH1*	1753	0.14	0.40	0.6	1.0
rs1801131	1	*MTHFR*	1710	0.28	0.34	0.6	1.0
rs12905663	15	*MTHFS*	1788	0.29	0.80	0.6	1.0
NA	1	*MTR_01_2_i_order*	1793	0.16	0.81	0.6	1.0
rs617219	5	*BHMT*	1790	0.32	0.94	0.6	1.0
rs12797843	11	*FOLH1*	1798	0.13	0.37	0.6	1.0
rs372447	15	*MTHFS*	1798	0.38	0.77	0.6	1.0
rs7253062	19	*DNMT1*	1798	0.38	1.00	0.6	1.0
rs11085720	19	*DNMT1*	1798	0.41	0.44	0.6	1.0
rs4911107	20	*DNMT3B*	1798	0.31	1.00	0.6	1.0
rs8101626	19	*DNMT1*	1798	0.39	0.89	0.6	1.0
rs6722613	2	*DNMT3A*	1798	0.40	0.89	0.7	1.0
rs4819130	21	*SLC19A1*	1794	0.45	0.15	0.7	1.0
rs4673965	2	*ATIC*	1798	0.40	0.94	0.7	1.0
rs2696935	11	*FOLH1*	1798	0.21	0.12	0.7	1.0
rs7111215	11	*FOLH1*	1774	0.40	0.52	0.7	1.0
rs750546	17	*PEMT*	1773	0.45	0.19	0.7	1.0
rs16853782	2	*ATIC*	1798	0.20	0.34	0.7	1.0
rs2288349	19	*DNMT1*	1797	0.38	0.78	0.7	1.0
rs1650697	5	*DHFR*	1791	0.23	0.06	0.7	1.0
rs12613	21	*CBS*	1793	0.09	0.83	0.7	1.0
rs2241807	3	*CHDH*	1798	0.42	0.89	0.7	1.0
rs1404774	2	*ATIC*	1784	0.22	0.42	0.7	1.0
rs3740392	10	*AS3MT*	1794	0.29	0.41	0.7	1.0
rs17291414	19	*DNMT1*	1798	0.28	0.73	0.7	1.0
rs8111085	19	*DNMT1*	1797	0.08	0.64	0.7	1.0
rs12462004	19	*DNMT1*	1795	0.08	0.64	0.7	1.0
rs10418707	19	*DNMT1*	1796	0.08	0.64	0.7	1.0
rs2116940	19	*DNMT1*	1797	0.08	0.64	0.7	1.0
rs8112895	19	*DNMT1*	1798	0.08	0.81	0.7	1.0
rs2288350	19	*DNMT1*	1798	0.08	0.64	0.7	1.0
rs10498034	2	*ATIC*	1798	0.16	0.90	0.7	1.0

Supplementary Table S4-6 (cont.) Corrected likelihood ratio test p-values of the interaction between LINE-1 methylation and SNPs in one-carbon metabolism pathway and bladder cancer susceptibility genes and in the SBS/EPICURO study

dbSNP (N=446)	Chromosome	Gene	N	MAF	*P* HWE	*P* LRT	Corrected *P* LRT
rs4804490	19	*DNMT1*	1796	0.08	0.64	0.7	1.0
rs11672909	19	*DNMT1*	1797	0.08	0.81	0.7	1.0
rs9305012	19	*DNMT1*	1797	0.08	0.64	0.7	1.0
rs4804125	19	*DNMT1*	1797	0.08	0.64	0.7	1.0
rs4804494	19	*DNMT1*	1797	0.08	0.64	0.7	1.0
rs2236224	14	*MTHFD1*	1798	0.36	0.07	0.7	1.0
rs8041943	15	*MTHFS*	1794	0.41	0.37	0.7	1.0
rs11191439	10	*AS3MT*	1795	0.12	0.75	0.7	1.0
rs2838977	21	*SLC19A1*	1796	0.40	0.72	0.7	1.0
rs4646341	17	*PEMT*	1795	0.37	0.56	0.7	1.0
rs8923	15	*MTHFS*	1798	0.08	0.15	0.7	1.0
rs1979277	17	*SHMT1*	1809	0.27	0.87	0.7	1.0
rs12591436	15	*MTHFS*	1798	0.35	0.55	0.7	1.0
rs11701960	21	*SLC19A1*	1797	0.18	0.91	0.7	1.0
rs2790	18	*TYMS*	1789	0.20	0.67	0.7	1.0
rs2838958	21	*SLC19A1*	1794	0.46	0.63	0.7	1.0
rs12614943	2	*ATIC*	1798	0.27	0.73	0.7	1.0
rs1917311	11	*FOLH1*	1753	0.40	0.72	0.7	1.0
rs4987173	6	*GNMT*	1798	0.50	0.46	0.7	1.0
rs515064	1	*CTH*	1793	0.35	0.50	0.7	1.0
rs767138	21	*SLC19A1*	1795	0.41	0.89	0.7	1.0
rs11158540	14	*MTHFD1*	1798	0.35	0.94	0.7	1.0
rs2586182	15	*MTHFS*	1798	0.14	0.78	0.7	1.0
rs11040416	11	*FOLH1*	1798	0.42	0.58	0.7	1.0
rs1256095	14	*MTHFD1*	1782	0.48	0.50	0.7	1.0
rs4673981	2	*ATIC*	1798	0.40	1.00	0.7	1.0
rs9282690	3	*ALDH1L1*	1793	0.08	1.00	0.7	1.0
rs11681447	2	*DNMT3A*	1796	0.29	0.62	0.7	1.0
rs12121543	1	*MTHFR*	1793	0.22	0.32	0.7	1.0
rs2865908	11	*FOLH1*	1798	0.18	0.50	0.7	1.0
rs4804122	19	*DNMT1*	1798	0.39	0.52	0.7	1.0
rs16853826	2	*ATIC*	1796	0.13	0.88	0.7	1.0
rs685487	15	*MTHFS*	1798	0.36	0.34	0.7	1.0
rs11158538	14	*MTHFD1*	1795	0.45	0.78	0.7	1.0
rs4094478	11	*FOLH1*	1770	0.20	0.34	0.7	1.0
rs3177999	21	*SLC19A1*	1790	0.46	0.17	0.7	1.0
rs11607791	11	*FOLH1*	1796	0.07	1.00	0.7	1.0
rs2877078	21	*SLC19A1*	1786	0.40	0.94	0.7	1.0
rs6058893	20	*DNMT3B*	1798	0.32	0.49	0.8	1.0
rs12987326	2	*DNMT3A*	1798	0.37	0.47	0.8	1.0

Supplementary Table S4-6 (cont.) Corrected likelihood ratio test p-values of the interaction between LINE-1 methylation and SNPs in one-carbon metabolism pathway and bladder cancer susceptibility genes and in the SBS/EPICURO study

dbSNP (N=446)	Chromosome	Gene	N	MAF	*P* HWE	*P* LRT	Corrected *P* LRT
rs3788200	21	*SLC19A1*	1798	0.45	0.31	0.8	1.0
rs4818789	21	*SLC19A1*	1798	0.25	0.06	0.8	1.0
rs8003567	14	*MTHFD1*	1798	0.11	0.47	0.8	1.0
rs2236222	14	*MTHFD1*	1798	0.10	0.27	0.8	1.0
rs11629135	14	*MTHFD1*	1797	0.11	0.38	0.8	1.0
rs16971249	15	*MTHFS*	1798	0.08	0.09	0.8	1.0
rs16906158	11	*FOLH1*	1796	0.08	1.00	0.8	1.0
rs2696923	11	*FOLH1*	1798	0.21	0.12	0.8	1.0
rs9890064	17	*PEMT*	1798	0.43	0.41	0.8	1.0
rs2586153	15	*MTHFS*	1777	0.15	0.59	0.8	1.0
rs6058869	20	*DNMT3B*	1797	0.33	0.76	0.8	1.0
rs1256142	14	*MTHFD1*	1798	0.44	0.10	0.8	1.0
rs1051266	21	*SLC19A1*	1798	0.45	0.20	0.8	1.0
rs7594432	2	*DNMT3A*	1798	0.44	0.13	0.8	1.0
rs12373907	21	*SLC19A1*	1797	0.38	0.20	0.8	1.0
rs2733106	15	*MTHFS*	1793	0.15	0.69	0.8	1.0
rs10769558	11	*FOLH1*	1797	0.21	0.18	0.8	1.0
rs282802	15	*MTHFS*	1798	0.29	0.09	0.8	1.0
rs4646340	17	*PEMT*	1798	0.37	0.51	0.8	1.0
rs10839210	11	*FOLH1*	1796	0.21	0.18	0.8	1.0
rs2162560	19	*DNMT1*	1797	0.38	0.94	0.8	1.0
rs6511677	19	*DNMT1*	1797	0.38	0.89	0.8	1.0
rs1081235	15	*MTHFS*	1798	0.20	0.67	0.8	1.0
rs17285431	15	*MTHFS*	1798	0.17	0.08	0.8	1.0
rs17751556	14	*MTHFD1*	1798	0.08	0.65	0.8	1.0
rs2424932	20	*DNMT3B*	1797	0.43	0.15	0.8	1.0
rs13401241	2	*DNMT3A*	1798	0.45	0.28	0.8	1.0
rs2289093	2	*DNMT3A*	1798	0.29	0.62	0.8	1.0
rs6801605	3	*CHDH*	1798	0.37	1.00	0.8	1.0
rs1888533	21	*SLC19A1*	1797	0.48	0.42	0.8	1.0
rs1051296	21	*SLC19A1*	1786	0.48	0.10	0.8	1.0
rs10839239	11	*FOLH1*	1795	0.23	0.85	0.8	1.0
rs11191457	10	*AS3MT*	1794	0.22	1.00	0.8	1.0
rs2586154	15	*MTHFS*	1798	0.14	0.89	0.8	1.0
rs1870576	15	*MTHFS*	1780	0.46	0.06	0.8	1.0
rs660439	11	*FOLH1*	1794	0.23	0.85	0.8	1.0
rs7085854	10	*AS3MT*	1797	0.22	0.85	0.8	1.0
rs17279753	15	*MTHFS*	1798	0.19	0.51	0.8	1.0
rs12438477	15	*MTHFS*	1797	0.36	0.61	0.8	1.0
rs12416687	10	*AS3MT*	1797	0.27	0.15	0.8	1.0

Supplementary Table S4-6 (cont.) Corrected likelihood ratio test p-values of the interaction between LINE-1 methylation and SNPs in one-carbon metabolism pathway and bladder cancer susceptibility genes and in the SBS/EPICURO study

dbSNP (N=446)	Chromosome	Gene	N	MAF	*P* HWE	*P* LRT	Corrected *P* LRT
rs559062	1	*CTH*	1793	0.22	0.85	0.8	1.0
rs7124266	11	*FOLH1*	1798	0.30	0.94	0.8	1.0
rs1051298	21	*SLC19A1*	1790	0.47	0.17	0.8	1.0
rs734693	2	*DNMT3A*	1795	0.29	0.51	0.8	1.0
rs1023159	21	*SLC19A1*	1796	0.42	0.78	0.8	1.0
rs11694842	2	*DNMT3A*	1797	0.28	0.36	0.8	1.0
rs2983733	14	*MTHFD1*	1798	0.44	0.68	0.8	1.0
rs12482346	21	*SLC19A1*	1797	0.48	0.10	0.8	1.0
rs567754	5	*BHMT*	1792	0.29	0.74	0.8	1.0
rs8011839	14	*MTHFD1*	1798	0.17	0.72	0.8	1.0
rs202712	11	*FOLH1*	1796	0.23	0.85	0.8	1.0
rs13002567	2	*DNMT3A*	1798	0.28	0.40	0.8	1.0
rs1473406	15	*MTHFS*	1796	0.15	0.79	0.8	1.0
rs3818239	14	*MTHFD1*	1787	0.13	0.66	0.8	1.0
rs7587636	2	*DNMT3A*	1798	0.45	0.25	0.8	1.0
rs4779165	15	*MTHFS*	1797	0.16	0.80	0.8	1.0
rs2035027	15	*MTHFS*	1798	0.16	0.80	0.8	1.0
rs16971450	15	*MTHFS*	1797	0.16	0.80	0.9	1.0
rs2983736	14	*MTHFD1*	1793	0.44	0.73	0.9	1.0
rs1076991	14	*MTHFD1*	1798	0.45	0.63	0.9	1.0
rs1369703	2	*DNMT3A*	1798	0.44	0.10	0.9	1.0
rs1046778	10	*AS3MT*	1798	0.32	0.70	0.9	1.0
rs1464864	2	*ATIC*	1798	0.30	0.81	0.9	1.0
rs7929543	11	*FOLH1*	1798	0.07	0.81	0.9	1.0
rs12999687	2	*DNMT3A*	1795	0.45	0.68	0.9	1.0
rs202700	11	*FOLH1*	1734	0.23	0.77	0.9	1.0
rs1808119	2	*ATIC*	1797	0.19	0.82	0.9	1.0
rs8018032	14	*MTHFD1*	1798	0.45	0.95	0.9	1.0
rs11677670	2	*DNMT3A*	1788	0.18	0.64	0.9	1.0
rs914231	21	*SLC19A1*	1791	0.45	0.19	0.9	1.0
rs11855092	15	*MTHFS*	1798	0.24	0.31	0.9	1.0
rs17279885	15	*MTHFS*	1798	0.20	0.67	0.9	1.0
rs2154583	21	*GART*	1791	0.40	0.67	0.9	1.0
rs16999714	19	*DNMT1*	1796	0.21	0.26	0.9	1.0
rs2733103	15	*MTHFS*	1797	0.15	0.51	0.9	1.0
rs3788190	21	*SLC19A1*	1795	0.47	0.10	0.9	1.0
rs9980967	21	*SLC19A1*	1797	0.11	0.38	0.9	1.0
rs6760069	2	*ATIC*	1797	0.15	0.35	0.9	1.0
rs9977111	21	*SLC19A1*	1750	0.33	0.14	0.9	1.0
rs6517178	21	*GART*	1798	0.40	0.72	0.9	1.0

Supplementary Table S4-6 (cont.) Corrected likelihood ratio test p-values of the interaction between LINE-1 methylation and SNPs in one-carbon metabolism pathway and bladder cancer susceptibility genes and in the SBS/EPICURO study

dbSNP (N=446)	Chromosome	Gene	N	MAF	*P* HWE	*P* LRT	Corrected *P* LRT
rs2987969	14	*MTHFD1*	1797	0.45	0.95	0.9	1.0
rs1917321	11	*FOLH1*	1790	0.50	0.54	0.9	1.0
rs8015278	14	*MTHFD1*	1798	0.07	0.18	0.9	1.0
rs7144437	14	*MTHFD1*	1797	0.07	0.10	0.9	1.0
rs4902278	14	*MTHFD1*	1791	0.07	0.18	0.9	1.0
rs8068641	17	*PEMT*	1792	0.11	0.62	0.9	1.0
rs11627525	14	*MTHFD1*	1798	0.11	0.08	0.9	1.0
rs6485991	11	*FOLH1*	1791	0.17	0.81	0.9	1.0
rs234706	21	*CBS*	1793	0.33	0.36	0.9	1.0
rs7215833	17	*PEMT*	1798	0.36	0.51	0.9	1.0
rs10932605	2	*ATIC*	1798	0.14	0.39	0.9	1.0
rs1164685	11	*FOLH1*	1793	0.38	0.47	0.9	1.0
rs4779140	15	*MTHFS*	1797	0.48	0.79	0.9	1.0
rs588458	11	*FOLH1*	1772	0.38	0.52	0.9	1.0
rs663649	1	*CTH*	1793	0.31	0.81	1.0	1.0
rs473334	1	*CTH*	1793	0.31	0.81	1.0	1.0
rs16853834	2	*ATIC*	1798	0.17	0.71	1.0	1.0
rs1846285	11	*FOLH1*	1795	0.16	0.54	1.0	1.0
rs914232	21	*SLC19A1*	1797	0.45	0.17	1.0	1.0
rs4144700	11	*FOLH1*	1798	0.38	0.47	1.0	1.0
rs2866358	11	*FOLH1*	1781	0.38	0.35	1.0	1.0
rs759920	19	*DNMT1*	1798	0.46	0.79	1.0	1.0
rs5749131	22	*TCN2*	1798	0.42	0.13	1.0	1.0
rs4819128	21	*SLC19A1*	1798	0.45	0.13	1.0	1.0
rs1888530	21	*SLC19A1*	1760	0.47	0.08	1.0	1.0
rs6087990	20	*DNMT3B*	1797	0.32	0.64	1.0	1.0
rs600671	15	*MTHFS*	1797	0.45	0.34	1.0	1.0
rs2290684	19	*DNMT1*	1797	0.46	0.79	1.0	1.0
rs898436	15	*MTHFS*	1793	0.45	0.37	1.0	1.0
rs749130	2	*DNMT3A*	1798	0.45	0.37	1.0	1.0
rs8003379	14	*MTHFD1*	1796	0.25	0.78	1.0	1.0
rs12148881	15	*MTHFS*	1797	0.26	0.19	1.0	1.0
rs1404772	2	*ATIC*	1798	0.08	0.34	1.0	1.0
rs865646	5	*DHFR*	1736	0.36	0.55	1.0	1.0
rs2834235	21	*GART*	1797	0.39	0.67	1.0	1.0
rs2424908	20	*DNMT3B*	1798	0.17	0.40	1.0	1.0
rs1074390	15	*MTHFS*	1798	0.38	0.77	1.0	1.0
rs8128676	21	*SLC19A1*	1754	0.22	0.92	1.0	1.0
rs663465	1	*CTH*	1792	0.42	0.36	1.0	1.0
rs2228611	19	*DNMT1*	1798	0.46	0.79	1.0	1.0

Supplementary Table S4-6 (cont.) Corrected likelihood ratio test p-values of the interaction between LINE-1 methylation and SNPs in one-carbon metabolism pathway and bladder cancer susceptibility genes and in the SBS/EPICURO study

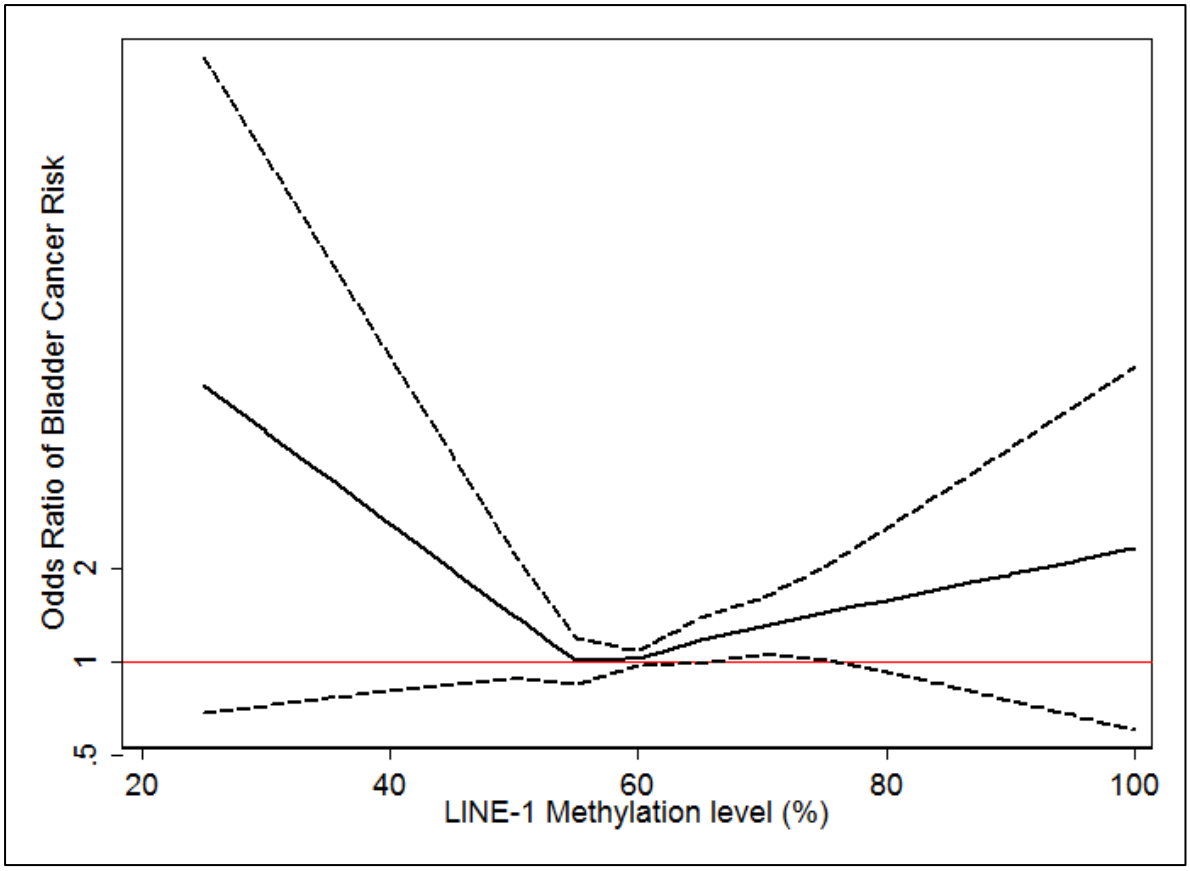
dbSNP (N=446)	Chromosome	Gene	N	MAF	*P* HWE	*P* LRT	Corrected *P* LRT
rs10420338	19	*DNMT1*	1798	0.47	0.64	1.0	1.0
rs6087988	20	*DNMT3B*	1798	0.19	1.00	1.0	1.0
rs2330183	21	*SLC19A1*	1757	0.45	0.05	1.0	1.0
rs12627639	21	*SLC19A1*	1797	0.21	0.68	1.0	1.0
rs282778	15	*MTHFS*	1798	0.25	0.86	1.0	1.0
rs376863	15	*MTHFS*	1772	0.50	0.73	1.0	1.0

N, number of subjects; MAF, minor allele frequency; *P* HWE - Hardy-Weinberg Equilibrium chi-square test *p*-value; *P* LRT, likelihood ratio test *p*-value for interaction; NA - dbSNP identifier not available

Supplementary Table S4-7 Comparisons of controls and cases with LINE-1 methylation data and without LINE-1 methylation data

Variables	Controls			Cases		
	With LINE-1 data, N=892	Without LINE-1 data, N=259	P-value	With LINE-1 data, N=952	Without LINE-1 data, N=219	P-value
Age in years, median (IQR)	66 (13)	69 (12)	0.001[a]	68 (13)	68 (13)	0.8*
Gender						
Male	792 (88.8)	217 (83.8)	0.03	826 (89.8)	202 (92.2)	0.03
Female	100 (11.2)	42 (16.2)		126 (13.2)	17 (7.8)	
Region						
Barcelona	168 (18.8)	50 (19.3)	0.2	162 (17.0)	46 (21.0)	0.5
Valles	135 (15.1)	33 (12.7)		148 (15.5)	36 (16.4)	
Elche	73 (8.2)	11 (4.3)		72 (7.6)	16 (7.3)	
Tenerife	145 (16.3)	43 (16.6)		173 (18.2)	31 (14.2)	
Asturias	371 (41.6)	122 (47.1)		397 (41.7)	90 (41.1)	
Smoking status						
Never	255 (28.7)	66 (26.1)	0.3	137 (14.5)	23 (10.6)	0.1
Occasional	66 (7.4)	27 (10.7)		34 (3.6)	12 (5.5)	
Former	329 (37.0)	97 (38.3)		376 (39.7)	77 (35.3)	
Current	239 (26.9)	63 (24.9)		399 (42.2)	106 (48.6)	
Missing	3	6		6	1	

*Mann–Whitney U test *p*-value.



Supplementary Figure S4-1 Results for urothelial carcinoma of the bladder risk and LINE-1 methylation level modeled as a continuous variable using restricted cubic spline regression in logistic regression model adjusted for age, gender, region and smoking status. The solid line shows the point estimates and the broken lines show the lower and upper 95% confidence intervals.

SUPPLEMENTARY MATERIALS CHAPTER V

Supplementary Table S5-1 Nonsignificant predictors of D4Z4 methylation level among the control population in the Spanish Bladder Cancer/EPICURO Study

Characteristic	N	D4Z4 methylation (%)				Unadjusted model				N	Adjusted model*			
		Mean	SD	Median	IQR	β	95% CI		P-value		β	95% CI		P-value
Age	718	—	—	—	—	0.01	-0.1	0.1	0.7	715	0.02	-0.1	0.1	0.6
Region														
Barcelona	130	61.9	11.0	64.1	12.5	Reference				130	Reference			
Valles	117	64.4	10.4	63.3	14.8	2.1	-0.4	4.6	0.1	117	1.9	-0.6	4.5	0.1
Elche	58	64.2	9.3	65.0	12.9	1.7	-1.4	4.8	0.3	58	1.8	-1.3	4.9	0.3
Tenerife	122	64.6	8.9	65.5	11.0	2.0	-0.4	4.5	0.1	121	2.0	-0.5	4.5	0.1
Asturias	291	63.6	9.6	64.2	14.1	1.1	-1.0	3.1	0.3	289	1.0	-1.0	3.1	0.3
Smoking status														
Never	206	63.7	9.9	64.4	12.7	Reference				206	Reference			
Occasional	53	64.7	8.1	64.8	9.1	0.7	-2.3	3.8	0.6	53	-0.2	-3.3	2.9	0.9
Former	261	63.7	9.6	64.5	14.7	-0.2	-2.0	1.7	0.9	261	-1.2	-3.2	0.8	0.3
Current	195	63.1	10.7	64.1	14.0	-0.4	-2.3	1.6	0.7	195	-1.3	-3.5	0.9	0.2
Body mass index†														
< 25.0	305	64.0	9.6	65.2	14.0	Reference				303	Reference			
25.0 - 29.9	215	64.2	9.3	65.3	11.8	0.5	-1.2	2.2	0.6	215	0.4	-1.4	2.1	0.7
≥ 30.0	40	60.2	12.8	62.1	18.5	-2.6	-5.8	0.7	0.1	40	-2.8	-6.1	0.5	0.09
Controls' diagnosis														
Hernia	278	64.1	10.0	64.4	13.1	Reference				276	Reference			
Fracture & Trauma	194	62.9	10.4	63.6	14.9	-1.2	-3.0	0.7	0.2	193	-0.9	-2.9	1.0	0.4
Hydrocele	107	63.5	9.3	65.0	11.8	-0.7	-3.0	1.5	0.5	107	-1.1	-3.5	1.3	0.4
Other Abdominal Surgery	84	64.4	8.9	65.2	12.2	0.1	-2.4	2.5	0.96	84	0.2	-2.2	2.7	0.8
Other Diseases	55	62.7	10.4	64.1	13.4	-1.0	-3.9	1.9	0.5	55	-0.8	-3.9	2.2	0.6
GSTM1														
GSTM1 (++/+-)	338	63.1	9.8	63.9	12.6	Reference				336	Reference			
GSTM1 (--)	373	64.0	10.0	65.1	14.3	0.8	-0.6	2.3	0.3	372	0.8	-0.7	2.3	0.3

Supplementary Table S5-1 (cont.) Nonsignificant predictors of D4Z4 methylation level among the control population in the Spanish Bladder Cancer/EPICURO Study

Characteristic	N	D4Z4 methylation (%)				Unadjusted model				N	Adjusted model*			
		Mean	SD	Median	IQR	β	95% CI		P-value		β	95% CI		P-value
GSTT1														
GSTT1 (++/+-)	561	63.5	10.0	64.5	13.5	Reference				558	Reference			
GSTT1 (--)	151	64.0	9.6	64.3	14.0	0.2	-1.6	2.0	0.8	151	0.3	-1.5	2.1	0.8
***NAT2* acetylator**														
Rapid/Intermediate	306	64.0	9.8	65.3	13.3	Reference				305	Reference			
Slow	409	63.2	9.9	63.8	13.3	-0.9	-2.3	0.6	0.3	407	-0.7	-2.2	0.8	0.4
Nutrient intake in µg/kcal/day														
Vitamin B_1	513	—	—	—	—	2.8	-2.2	7.8	0.3	512	2.7	-2.6	8.0	0.3
Vitamin B_2	513	—	—	—	—	-0.2	-3.3	2.9	0.9	512	-0.5	-3.7	2.7	0.8
Vitamin B_3	513	—	—	—	—	0.1	-0.2	0.4	0.6	512	0.1	-0.2	0.4	0.6
Vitamin B_6	513	—	—	—	—	1.4	-2.1	4.9	0.4	512	1.1	-2.7	4.8	0.6
Vitamin B_{12}	513	—	—	—	—	0.04	-0.2	0.3	0.7	512	0.1	-0.2	0.4	0.6
Folate	513	—	—	—	—	0.01	-0.01	0.02	0.3	512	0.01	-0.01	0.02	0.3
Protein	513	—	—	—	—	-0.004	-0.1	0.1	0.9	512	-0.01	-0.1	0.1	0.9
Alcohol	513	—	—	—	—	0.02	-0.1	0.1	0.7	512	0.04	-0.1	0.1	0.5
Fruit and vegetables in g/day/kcal														
Fruit	508	—	—	—	—	0.0005	-0.01	0.01	0.9	507	-0.001	-0.01	0.01	0.9
Vegetables	508	—	—	—	—	0.003	-0.01	0.01	0.5	507	0.004	-0.01	0.01	0.4
Fruit and vegetables combined	507	—	—	—	—	0.001	-0.004	0.01	0.7	506	0.001	-0.004	0.01	0.8
Toenail trace elements in µg/g														
Aluminum	520	—	—	—	—	0.01	-0.01	0.04	0.3	520	0.01	-0.02	0.04	0.5
Arsenic	521	—	—	—	—	11.5	-1.9	24.9	0.09	521	13.3	-0.9	27.4	0.07
Cadmium	521	—	—	—	—	-0.2	-2.0	1.6	0.8	521	-0.2	-2.1	1.6	0.8
Chromium	520	—	—	—	—	0.1	-0.2	0.5	0.4	520	0.2	-0.2	0.5	0.3
Copper	521	—	—	—	—	-0.3	-0.6	0.1	0.1	521	-0.3	-0.6	0.1	0.1

Supplementary Table S5-1 (cont.) Nonsignificant predictors of D4Z4 methylation level among the control population in the Spanish Bladder Cancer/EPICURO Study

Characteristic	N	D4Z4 methylation (%)				Unadjusted model				N	Adjusted model*			
		Mean	SD	Median	IQR	β	95% CI		P-value		β	95% CI		P-value
Toenail trace elements in µg/g														
Iron	519	—	—	—	—	0.01	-0.01	0.03	0.2	519	0.004	-0.002	0.01	0.2
Lead	521	—	—	—	—	-0.04	-0.5	0.4	0.9	521	-0.01	-0.4	0.4	0.96
Manganese	521	—	—	—	—	0.3	-0.3	0.8	0.3	521	0.2	-0.3	0.8	0.4
Nickel	521	—	—	—	—	0.01	-0.04	0.05	0.7	521	0.01	-0.04	0.05	0.8
Vanadium	513	—	—	—	—	3.3	-5.5	12.0	0.5	513	2.5	-6.6	11.6	0.6
Zinc	521	—	—	—	—	-0.01	-0.02	0.003	0.2	521	-0.01	-0.02	0.005	0.2

SD, standard deviation; IQR, interquartile range.

*Adjusted for age, gender, region, and smoking status (never, occasional, former, and current smoker). Beta coefficients of tobacco type are adjusted for age, gender and region.

†Body mass index is the weight in kilograms divided by the square of the height in meters.

Supplementary Table S5-2 Predictors of D4Z4 methylation level among the control population in the Spanish Bladder Cancer/EPICURO Study

Characteristic	Adjusted model*				
	N	β	95% CI		P-value
Age	718	0.03	-0.05	0.1	0.5
Gender					
Male	642	Reference			
Female	76	-2.5	-4.9	-0.1	0.04
Region					
Barcelona	130	Reference			
Valles	117	2.0	-0.5	4.5	0.1
Elche	58	1.9	-1.2	5.0	0.2
Tenerife	122	2.0	-0.4	4.5	0.1
Asturias	291	1.1	-1.0	3.2	0.3
Smoking status					
Never	206	Reference			
Occasional	53	-0.2	-3.3	2.9	0.9
Former	261	-1.2	-3.2	0.8	0.3
Current	195	-1.3	-3.5	0.9	0.2
Tobacco type					
Never	206	Reference			
Blond tobacco only	82	-3.7	-6.5	-0.9	0.01
Black tobacco only	171	-0.4	-2.7	1.8	0.7
Both types	126	-0.9	-3.3	1.6	0.5
Unknown	78	-2.2	-5.0	0.6	0.1
Body mass index†					
< 25.0	305	Reference			
25.0 - 29.9	215	0.4	-1.3	2.2	0.7
≥ 30.0	40	-2.5	-5.8	0.8	0.1
Controls' diagnosis					
Hernia	278	Reference			
Fracture & Trauma	194	-0.9	-2.9	1.1	0.4
Hydrocele	107	-1.1	-3.5	1.3	0.4
Other Abdominal Surgery	84	0.1	-2.4	2.6	0.9
Other Diseases	55	-0.9	-3.9	2.2	0.6
GSTM1					
GSTM1 (++/+-)	338	Reference			
GSTM1 (--)	373	0.7	-0.7	2.2	0.3
GSTT1					
GSTT1 (++/+-)	561	Reference			
GSTT1 (--)	151	0.2	-1.6	2.0	0.8
***NAT2* acetylator**					
Rapid/Intermediate	306	Reference			
Slow	409	-0.7	-2.2	0.8	0.4

Supplementary Table S5-2 (cont.) Predictors of D4Z4 methylation level among the control population in the Spanish Bladder Cancer/EPICURO Study

Characteristic	Adjusted model*				
	N	β	95% CI		P-value
Nutrient intake in µg/kcal/day					
Vitamin B_1	513	2.9	-2.4	8.2	0.3
Vitamin B_2	513	-0.4	-3.7	2.8	0.8
Vitamin B_3	513	0.1	-0.2	0.4	0.5
Vitamin B_6	513	1.5	-2.2	5.3	0.4
Vitamin B_{12}	513	0.1	-0.2	0.3	0.7
Folate	513	0.01	-0.01	0.02	0.2
Protein	513	0.002	-0.1	0.1	0.96
Alcohol	513	0.02	-0.1	0.1	0.7
Fruit and vegetables in g/day/kcal					
Fruit	508	0.00001	-0.01	0.01	0.99
Vegetables	508	0.004	-0.005	0.01	0.4
Fruit and vegetables combined	507	0.001	-0.004	0.01	0.7
Toenail trace elements in µg/g					
Aluminum	520	0.01	-0.02	0.04	0.5
Arsenic	521	13.0	-1.1	27.1	0.07
Cadmium	521	-0.3	-2.1	1.6	0.8
Chromium	520	0.2	-0.2	0.5	0.3
Copper	521	-0.3	-0.6	0.1	0.1
Iron	519	0.004	-0.002	0.01	0.2
Lead	521	-0.01	-0.4	0.4	0.97
Manganese	521	0.3	-0.3	0.8	0.4
Nickel	521	0.01	-0.04	0.05	0.8
Selenium	521	-7.9	-12.5	-3.4	0.0006
Vanadium	513	2.6	-6.5	11.7	0.6
Zinc	521	-0.01	-0.02	0.004	0.2

*Adjusted for age, gender, and region.

†Body mass index is the weight in kilograms divided by the square of the height in meters.

Supplementary Table S5-3 Association of each individual D4Z4 CpG site methylation level and risk of bladder cancer in the Spanish Bladder Cancer/EPICURO Study

D4Z4 methylation	Cases	Controls	OR*	95% CI		P-value
D4Z4-CpG1						
M1, < 70.1%	316	358	1 Reference			
M2, ≥ 70.1%	385	357	1.26	1.01	1.57	0.04
D4Z4-CpG2						
M1, < 59.6%	310	358	1 Reference			
M2, ≥ 59.6%	391	357	1.33	1.07	1.66	0.01
D4Z4-CpG3						
M1, < 63.9%	331	356	1 Reference			
M2, ≥ 63.9%	370	359	1.13	0.90	1.40	0.3
D4Z4-CpG4						
M1, < 55.5%	343	358	1 Reference			
M2, ≥ 55.5%	358	357	1.08	0.87	1.35	0.5
D4Z4-CpG5						
M1, < 64.8%	323	357	1 Reference			
M2, ≥ 64.8%	378	358	1.20	0.96	1.50	0.1
D4Z4-CpG6						
M1, < 71.9%	325	358	1 Reference			
M2, ≥ 71.9%	376	357	1.21	0.97	1.50	0.09

M1, D4Z4 methylation level less than the median; M2, D4Z4 methylation level greater than or equal to the median.

*Adjusted for age, gender, region, and smoking status.

Supplementary Table S5-4 Association between D4Z4 methylation level and urothelial carcinoma of the bladder risk, by age groups, gender, smoking status, and tobacco type in the Spanish Bladder Cancer/EPICURO Study

D4Z4 Methylation	Cases	Controls	OR	95% CI		P-value	P-interaction
Age*							
Age <60 years							
M1, < 64.4%	77	102	1 Reference				
M2, ≥ 64.4%	74	100	1.04	0.67	1.62	0.9	
Age 60-69 years							
M1, < 64.4%	127	139	1 Reference				
M2, ≥ 64.4%	134	146	1.08	0.75	1.56	0.7	
Age 70+ years							
M1, < 64.4%	120	117	1 Reference				
M2, ≥ 64.4%	169	111	1.61	1.11	2.33	0.01	0.2
Gender†							
Males							
M1, < 64.4%	273	309	1 Reference				
M2, ≥ 64.4%	338	330	1.20	0.95	1.52	0.1	
Females							
M1, < 64.4%	51	49	1 Reference				
M2, ≥ 64.4%	39	27	1.35	0.69	2.62	0.4	0.6
Smoking status‡							
Never							
M1, < 64.4%	52	103	1 Reference				
M2, ≥ 64.4%	50	103	1.25	0.75	2.09	0.4	
Occasional							
M1, < 64.4%	12	25	1 Reference				
M2, ≥ 64.4%	18	28	0.94	0.31	2.80	0.9	
Former							
M1, < 64.4%	116	129	1 Reference				
M2, ≥ 64.4%	150	132	1.27	0.89	1.80	0.2	
Current							
M1, < 64.4%	144	101	1 Reference				
M2, ≥ 64.4%	159	94	1.16	0.80	1.68	0.4	0.98
Tobacco type§							
Never smoker							
M1, < 64.4%	52	103	1 Reference				
M2, ≥ 64.4%	50	103	1.25	0.75	2.09	0.4	
Blond tobacco only							
M1, < 64.4%	25	46	1 Reference				
M2, ≥ 64.4%	29	36	1.50	0.71	3.17	0.3	
Black tobacco only							
M1, < 64.4%	105	83	1 Reference				
M2, ≥ 64.4%	134	87	1.20	0.80	1.80	0.4	

Supplementary Table S5-4 (cont.) Association between D4Z4 methylation level and urothelial carcinoma of the bladder risk, by age groups, gender, smoking status, and tobacco type in the Spanish Bladder Cancer/EPICURO Study

D4Z4 Methylation	Cases	Controls	OR	95% CI		P-value	P-interaction
Tobacco type§							
Both types							
M1, < 64.4%	86	57	1 Reference				
M2, ≥ 64.4%	97	69	0.93	0.58	1.49	0.8	
Unknown							
M1, < 64.4%	44	44	1 Reference				
M2, ≥ 64.4%	49	34	1.47	0.78	2.75	0.2	0.7

M1, D4Z4 methylation level less than the median; M2, D4Z4 methylation level greater than or equal to the median.

*Adjusted for gender, region, and smoking status (never, occasional, former, and current smoker).

†Adjusted for age, region, and smoking status (never, occasional, former, and current smoker).

‡Data were adjusted for age, gender and region.

§Adjusted for age, gender, region, and smoking status (never, occasional, former, and current smoker).

Note: No interaction was observed between D4Z4 methylation and number of cigarettes smoked per day (P-interaction = 0.4), duration of cigarette smoking in years (P-interaction = 1.0), and pack-years (P-interaction = 0.4) with effect on urothelial carcinoma of the bladder risk.

Supplementary Table S5-5 Association between D4Z4 methylation level and urothelial carcinoma of the bladder risk, by nutrient, fruit and vegetable intake, and trace elements in the Spanish Bladder Cancer/EPICURO Study

Characteristic	P-interaction*
Nutrients in µg/kcal/day	
Vitamin B_1	0.9
Vitamin B_2	0.6
Vitamin B_3	0.6
Vitamin B_6	0.8
Vitamin B_{12}	0.98
Folate	0.7
Protein	0.4
Alcohol	0.8
Fruit and vegetables in g/day/kcal	
Fruits	0.7
Vegetables	0.98
Fruit and vegetables	0.8
Toenail trace elements in µg/g	
Aluminum	0.1
Arsenic	0.1
Cadmium	0.6
Chromium	0.6
Copper	0.2
Lead	0.3
Nickel	0.3
Selenium	0.4
Vanadium	0.1

*Interaction p-value calculated from logistic regression models adjusted for age, gender, region, and smoking status.

Supplementary Table S5-6 Uncorrected and corrected p-values for the interaction between D4Z4 methylation levels and selected SNPs in the one-carbon metabolism pathway in the Spanish Bladder Cancer/EPICURO Study

SNPs modeled in additive mode of inheritance							
dbSNP ID (N=496)	Chromosome	Gene	N	MAF	*P* HWE	*P* LRT	Corrected *P* LRT
rs11040387	11	*FOLH1*	1379	0.07	0.4	0.0002	0.05
rs7358352	11	*FOLH1*	1387	0.09	0.4	0.001	0.2
rs12098986	11	*FOLH1*	1386	0.09	0.2	0.001	0.2
rs632220	11	*FOLH1*	1388	0.09	0.4	0.001	0.3
rs598841	11	*FOLH1*	1383	0.09	0.2	0.001	0.3
rs11040261	11	*FOLH1*	1388	0.09	0.4	0.001	0.3
rs1819409	11	*FOLH1*	1376	0.09	0.3	0.001	0.3
rs12293923	11	*FOLH1*	1383	0.09	0.2	0.001	0.3
rs650826	11	*FOLH1*	1386	0.09	0.2	0.001	0.3
rs683680	11	*FOLH1*	1387	0.09	0.2	0.002	0.4
rs7117025	11	*FOLH1*	1388	0.09	0.4	0.002	0.4
rs7926211	11	*FOLH1*	1385	0.09	0.2	0.002	0.4
rs11040263	11	*FOLH1*	1385	0.09	0.2	0.003	0.5
rs4495895	11	*FOLH1*	1353	0.09	0.4	0.005	0.7
rs2865908	11	*FOLH1*	1388	0.19	0.9	0.006	0.7
rs3821353	2	*ATIC*	1388	0.21	0.4	0.006	0.8
rs2236224	14	*MTHFD1*	1388	0.36	0.1	0.007	0.8
rs4094478	11	*FOLH1*	1365	0.20	0.7	0.007	0.8
rs6495449	15	*MTHFS*	1388	0.11	0.09	0.008	0.8
rs7929543	11	*FOLH1*	1388	0.08	1.0	0.009	0.9
rs10839210	11	*FOLH1*	1386	0.21	0.5	0.009	0.9
rs10769558	11	*FOLH1*	1388	0.21	0.5	0.009	0.9
rs1847638	11	*FOLH1*	1335	0.21	1.0	0.01	0.9
rs16853782	2	*ATIC*	1388	0.21	0.7	0.01	0.9
rs6058869	20	*DNMT3B*	1388	0.33	0.7	0.01	0.9
rs1256142	14	*MTHFD1*	1388	0.44	0.07	0.01	0.9
rs11191457	10	*AS3MT*	1385	0.23	0.4	0.01	0.9
rs3772078	2	*ATIC*	1388	0.21	0.6	0.01	0.9
rs6087990	20	*DNMT3B*	1388	0.33	0.6	0.01	0.9
rs4441015	11	*FOLH1*	1353	0.15	0.5	0.02	1.0
rs7085854	10	*AS3MT*	1387	0.23	0.6	0.02	1.0
rs2696923	11	*FOLH1*	1388	0.21	0.3	0.02	1.0
rs6058893	20	*DNMT3B*	1388	0.33	0.2	0.02	1.0
rs11607791	11	*FOLH1*	1387	0.07	1.0	0.02	1.0
rs7124266	11	*FOLH1*	1388	0.30	0.6	0.02	1.0
rs11040198	11	*FOLH1*	1381	0.21	0.2	0.02	1.0
rs2305230	3	*ALDH1L1*	1387	0.19	0.7	0.02	1.0
rs2696935	11	*FOLH1*	1388	0.21	0.4	0.02	1.0

Supplementary Table S5-6 (cont.) Uncorrected and corrected p-values for the interaction between D4Z4 methylation levels and selected SNPs in the one-carbon metabolism pathway in the Spanish Bladder Cancer/EPICURO Study

dbSNP ID (N=496)	Chromosome	Gene	N	MAF	*P* HWE	*P* LRT	Corrected *P* LRT
rs7111711	11	*FOLH1*	1388	0.25	0.9	0.02	1.0
rs10839296	11	*FOLH1*	1371	0.25	0.8	0.02	1.0
rs2834235	21	*GART*	1388	0.38	0.7	0.02	1.0
rs4817577	21	*GART*	1388	0.34	0.9	0.02	1.0
rs7107178	11	*FOLH1*	1387	0.25	1.0	0.02	1.0
rs11040421	11	*FOLH1*	1388	0.15	0.6	0.02	1.0
rs4817579	21	*GART*	1388	0.35	1.0	0.03	1.0
rs7283354	21	*GART*	1388	0.34	0.9	0.03	1.0
rs4911107	20	*DNMT3B*	1388	0.32	0.6	0.03	1.0
rs585800	5	*BHMT*	1388	0.26	0.05	0.03	1.0
rs2236225	14	*MTHFD1*	1387	0.43	0.2	0.04	1.0
rs10839229	11	*FOLH1*	1386	0.08	0.3	0.04	1.0
rs865646	5	*DHFR*	1340	0.34	0.4	0.04	1.0
rs10839224	11	*FOLH1*	1388	0.08	0.3	0.04	1.0
rs2838958	21	*SLC19A1*	1385	0.45	0.7	0.04	1.0
rs2154583	21	*GART*	1384	0.39	0.7	0.04	1.0
rs11158542	14	*MTHFD1*	1388	0.29	0.8	0.05	1.0
rs6517178	21	*GART*	1388	0.39	0.7	0.05	1.0
rs16906158	11	*FOLH1*	1387	0.08	0.6	0.05	1.0
rs12438477	15	*MTHFS*	1387	0.36	0.4	0.05	1.0
rs473334	1	*CTH*	1388	0.31	0.9	0.05	1.0
rs16853826	2	*ATIC*	1386	0.13	0.9	0.05	1.0
rs663649	1	*CTH*	1388	0.31	0.9	0.05	1.0
rs4779140	15	*MTHFS*	1387	0.48	0.6	0.05	1.0
rs7560488	2	*DNMT3A*	1333	0.47	0.2	0.05	1.0
rs3862342	11	*FOLH1*	1386	0.28	0.8	0.06	1.0
rs11040432	11	*FOLH1*	1387	0.08	1.0	0.06	1.0
rs34033751	11	*FOLH1*	1371	0.11	0.4	0.06	1.0
rs6573559	14	*MTHFD1*	1388	0.29	1.0	0.06	1.0
rs7587636	2	*DNMT3A*	1388	0.45	0.1	0.06	1.0
rs6141813	20	*DNMT3B*	1388	0.13	0.8	0.06	1.0
rs9974061	21	*SLC19A1*	1388	0.18	1.0	0.06	1.0
rs11627387	14	*MTHFD1*	1388	0.29	0.9	0.07	1.0
rs13401241	2	*DNMT3A*	1388	0.45	0.1	0.07	1.0
rs9323450	14	*MTHFD1*	1388	0.30	0.8	0.07	1.0
rs11634787	15	*MTHFS*	1388	0.09	0.3	0.07	1.0
rs12913164	15	*MTHFS*	1388	0.07	0.8	0.07	1.0
rs2834233	21	*GART*	1387	0.08	0.5	0.08	1.0
rs4911263	20	*DNMT3B*	1388	0.32	1.0	0.08	1.0
rs2281603	14	*MTHFD1*	1388	0.19	0.2	0.08	1.0

Supplementary Table S5-6 (cont.) Uncorrected and corrected p-values for the interaction between D4Z4 methylation levels and selected SNPs in the one-carbon metabolism pathway in the Spanish Bladder Cancer/EPICURO Study

dbSNP ID (N=496)	Chromosome	Gene	N	MAF	*P* HWE	*P* LRT	Corrected *P* LRT
rs12903985	15	*MTHFS*	1388	0.28	0.4	0.09	1.0
rs1164681	11	*FOLH1*	1388	0.12	0.5	0.09	1.0
rs7934591	11	*FOLH1*	1388	0.08	0.8	0.09	1.0
rs2424921	20	*DNMT3B*	1388	0.39	0.3	0.09	1.0
rs2424932	20	*DNMT3B*	1387	0.43	0.5	0.09	1.0
rs8659	5	*MTRR*	1386	0.35	0.3	0.1	1.0
rs11040353	11	*FOLH1*	1387	0.08	0.8	0.1	1.0
rs7113075	11	*FOLH1*	1387	0.08	0.8	0.1	1.0
rs10418	22	*TCN2*	1367	0.21	0.7	0.1	1.0
rs7563206	2	*ATIC*	1387	0.47	0.6	0.1	1.0
rs6058891	20	*DNMT3B*	1386	0.39	0.2	0.1	1.0
rs2035027	15	*MTHFS*	1388	0.16	0.5	0.1	1.0
rs7220132	17	*PEMT*	1388	0.29	0.7	0.1	1.0
rs2424928	20	*DNMT3B*	1388	0.39	0.2	0.1	1.0
rs4646410	17	*PEMT*	1384	0.31	0.9	0.1	1.0
rs8074074	17	*PEMT*	1386	0.29	0.8	0.1	1.0
rs12900076	15	*MTHFS*	1385	0.08	0.6	0.1	1.0
rs3897953	15	*MTHFS*	1388	0.09	0.8	0.1	1.0
rs11040390	11	*FOLH1*	1388	0.08	1.0	0.1	1.0
rs6706415	2	*ATIC*	1388	0.30	0.4	0.1	1.0
rs12222221	11	*FOLH1*	1388	0.08	1.0	0.1	1.0
rs6058897	20	*DNMT3B*	1388	0.43	0.2	0.1	1.0
rs12592743	15	*MTHFS*	1388	0.09	0.5	0.1	1.0
rs2424922	20	*DNMT3B*	1387	0.39	0.2	0.1	1.0
rs1846285	11	*FOLH1*	1385	0.17	0.2	0.1	1.0
rs6058883	20	*DNMT3B*	1387	0.39	0.3	0.1	1.0
rs4779165	15	*MTHFS*	1387	0.16	0.4	0.1	1.0
rs910085	20	*DNMT3B*	1387	0.29	0.5	0.1	1.0
rs4479310	17	*PEMT*	1388	0.29	0.6	0.1	1.0
rs2424914	20	*DNMT3B*	1388	0.39	0.4	0.1	1.0
rs16971450	15	*MTHFS*	1387	0.16	0.4	0.1	1.0
rs6495441	15	*MTHFS*	1388	0.24	0.3	0.1	1.0
rs1880580	15	*MTHFS*	1388	0.30	0.09	0.1	1.0
rs9910747	17	*PEMT*	1388	0.07	0.4	0.1	1.0
rs2838956	21	*SLC19A1*	1373	0.44	0.07	0.1	1.0
rs7946	17	*PEMT*	1387	0.29	0.4	0.1	1.0
rs559062	1	*CTH*	1388	0.22	0.6	0.1	1.0
rs663465	1	*CTH*	1387	0.41	0.4	0.1	1.0
rs9890064	17	*PEMT*	1388	0.43	0.4	0.1	1.0
rs1046778	10	*AS3MT*	1388	0.33	0.8	0.1	1.0

Supplementary Table S5-6 (cont.) Uncorrected and corrected p-values for the interaction between D4Z4 methylation levels and selected SNPs in the one-carbon metabolism pathway in the Spanish Bladder Cancer/EPICURO Study

dbSNP ID (N=496)	Chromosome	Gene	N	MAF	*P* HWE	*P* LRT	Corrected *P* LRT
rs1880586	2	*ATIC*	1387	0.47	0.6	0.2	1.0
rs1404772	2	*ATIC*	1388	0.08	0.4	0.2	1.0
rs6087988	20	*DNMT3B*	1388	0.20	0.6	0.2	1.0
rs3760188	17	*PEMT*	1388	0.46	0.6	0.2	1.0
rs9332	5	*MTRR*	1388	0.12	0.2	0.2	1.0
rs4819128	21	*SLC19A1*	1388	0.44	0.2	0.2	1.0
rs12898642	15	*MTHFS*	1387	0.43	0.2	0.2	1.0
rs4818789	21	*SLC19A1*	1388	0.25	0.5	0.2	1.0
rs4646344	17	*PEMT*	1388	0.47	0.7	0.2	1.0
rs16988828	22	*TCN2*	1388	0.09	0.2	0.2	1.0
rs7102641	11	*FOLH1*	1385	0.08	0.4	0.2	1.0
rs1074390	15	*MTHFS*	1388	0.37	0.6	0.2	1.0
rs6749992	2	*DNMT3A*	1388	0.47	0.8	0.2	1.0
rs7605753	2	*DNMT3A*	1388	0.47	0.6	0.2	1.0
rs3755817	3	*CHDH*	1387	0.30	0.5	0.2	1.0
rs10179873	2	*ATIC*	1388	0.30	0.4	0.2	1.0
rs1051298	21	*SLC19A1*	1387	0.46	0.3	0.2	1.0
rs13317328	3	*CHDH*	1388	0.10	0.1	0.2	1.0
rs11852515	15	*MTHFS*	1388	0.12	0.7	0.2	1.0
rs1983462	2	*ATIC*	1388	0.30	0.3	0.2	1.0
rs1380642	15	*MTHFS*	1388	0.18	0.3	0.2	1.0
NA	1	*MTR_01_OR*	1389	0.16	0.7	0.2	1.0
rs2066470	1	*MTHFR*	1384	0.09	0.2	0.2	1.0
rs2424913	20	*DNMT3B*	1387	0.37	0.2	0.2	1.0
rs4911108	20	*DNMT3B*	1384	0.29	0.7	0.2	1.0
rs2372535	2	*ATIC*	1388	0.15	1.0	0.2	1.0
rs10197559	2	*ATIC*	1380	0.29	0.9	0.2	1.0
rs1888530	21	*SLC19A1*	1358	0.47	0.07	0.2	1.0
rs202676	11	*FOLH1*	1380	0.17	0.8	0.2	1.0
rs2424906	20	*DNMT3B*	1388	0.38	0.2	0.2	1.0
rs4646404	17	*PEMT*	1383	0.36	0.3	0.2	1.0
rs2115536	15	*MTHFS*	1388	0.50	0.05	0.2	1.0
rs12416687	10	*AS3MT*	1388	0.28	0.06	0.2	1.0
rs10380	5	*MTRR*	1388	0.10	0.4	0.2	1.0
rs515064	1	*CTH*	1388	0.36	0.8	0.2	1.0
rs11892429	2	*ATIC*	1388	0.29	0.7	0.2	1.0
rs9282690	3	*ALDH1L1*	1388	0.07	0.8	0.2	1.0
rs914232	21	*SLC19A1*	1387	0.44	0.2	0.2	1.0
rs10197653	2	*ATIC*	1388	0.29	0.1	0.2	1.0
rs3788200	21	*SLC19A1*	1388	0.45	0.3	0.2	1.0

Supplementary Table S5-6 (cont.) Uncorrected and corrected p-values for the interaction between D4Z4 methylation levels and selected SNPs in the one-carbon metabolism pathway in the Spanish Bladder Cancer/EPICURO Study

dbSNP ID (N=496)	Chromosome	Gene	N	MAF	*P* HWE	*P* LRT	Corrected *P* LRT
rs1950902	14	*MTHFD1*	1388	0.14	0.4	0.2	1.0
rs6711622	2	*DNMT3A*	1388	0.44	1.0	0.2	1.0
rs35020344	14	*MTHFD1*	1387	0.47	0.7	0.2	1.0
rs914231	21	*SLC19A1*	1383	0.44	0.2	0.2	1.0
rs16971253	15	*MTHFS*	1387	0.10	1.0	0.2	1.0
rs3788190	21	*SLC19A1*	1387	0.47	0.1	0.2	1.0
rs11677670	2	*DNMT3A*	1382	0.17	0.5	0.3	1.0
rs4646359	17	*PEMT*	1388	0.45	0.3	0.3	1.0
rs770144	15	*MTHFS*	1388	0.20	0.3	0.3	1.0
rs12482346	21	*SLC19A1*	1388	0.47	0.1	0.3	1.0
rs1051266	21	*SLC19A1*	1388	0.45	0.2	0.3	1.0
rs1917311	11	*FOLH1*	1353	0.40	0.4	0.3	1.0
rs8074191	17	*PEMT*	1372	0.28	0.9	0.3	1.0
rs7215833	17	*PEMT*	1388	0.35	0.5	0.3	1.0
rs2115540	15	*MTHFS*	1387	0.50	0.05	0.3	1.0
rs17745484	2	*DNMT3A*	1387	0.35	0.8	0.3	1.0
rs7111215	11	*FOLH1*	1368	0.40	0.2	0.3	1.0
rs7117247	11	*FOLH1*	1388	0.07	0.1	0.3	1.0
rs8041943	15	*MTHFS*	1385	0.42	0.6	0.3	1.0
rs2838964	21	*SLC19A1*	1388	0.07	1.0	0.3	1.0
rs9897362	17	*PEMT*	1388	0.06	0.3	0.3	1.0
rs2330183	21	*SLC19A1*	1358	0.44	0.05	0.3	1.0
rs679470	11	*FOLH1*	1387	0.17	0.6	0.3	1.0
rs740234	22	*TCN2*	1388	0.23	0.8	0.3	1.0
rs282778	15	*MTHFS*	1388	0.27	0.8	0.3	1.0
rs10400277	11	*FOLH1*	1368	0.13	0.6	0.3	1.0
rs1805087	1	*MTR*	1388	0.17	0.7	0.3	1.0
rs17279286	15	*MTHFS*	1387	0.05	0.7	0.3	1.0
rs1051296	21	*SLC19A1*	1375	0.47	0.1	0.3	1.0
rs12613	21	*CBS*	1388	0.08	1.0	0.3	1.0
rs7583409	2	*DNMT3A*	1387	0.35	0.4	0.3	1.0
rs6495446	15	*MTHFS*	1387	0.26	0.1	0.3	1.0
rs12999687	2	*DNMT3A*	1386	0.45	0.8	0.3	1.0
rs11085720	19	*DNMT1*	1388	0.42	0.5	0.3	1.0
rs10519256	15	*MTHFS*	1388	0.11	0.1	0.3	1.0
rs3740392	10	*AS3MT*	1385	0.29	0.1	0.3	1.0
rs34048824	2	*DNMT3A*	1387	0.50	1.0	0.3	1.0
rs7253062	19	*DNMT1*	1388	0.39	0.7	0.3	1.0
rs4819130	21	*SLC19A1*	1384	0.45	0.2	0.3	1.0
rs2290684	19	*DNMT1*	1388	0.47	0.8	0.3	1.0

Supplementary Table S5-6 (cont.) Uncorrected and corrected p-values for the interaction between D4Z4 methylation levels and selected SNPs in the one-carbon metabolism pathway in the Spanish Bladder Cancer/EPICURO Study

dbSNP ID (N=496)	Chromosome	Gene	N	MAF	*P* HWE	*P* LRT	Corrected *P* LRT
rs1667627	14	*MTHFD2*	1387	0.46	0.2	0.3	1.0
rs7217764	17	*PEMT*	1388	0.05	1.0	0.3	1.0
rs282795	15	*MTHFS*	1387	0.32	0.7	0.3	1.0
rs4646341	17	*PEMT*	1385	0.36	0.5	0.3	1.0
rs4804122	19	*DNMT1*	1388	0.40	0.4	0.3	1.0
rs10163099	15	*MTHFS*	1385	0.26	0.1	0.3	1.0
rs588458	11	*FOLH1*	1367	0.38	0.3	0.3	1.0
rs1164685	11	*FOLH1*	1385	0.38	0.3	0.3	1.0
rs4532960	10	*AS3MT*	1387	0.44	0.3	0.3	1.0
rs1256107	14	*MTHFD1*	1387	0.48	0.7	0.3	1.0
rs3177999	21	*SLC19A1*	1380	0.45	0.2	0.3	1.0
rs12905663	15	*MTHFS*	1381	0.27	0.2	0.3	1.0
rs10748835	10	*AS3MT*	1388	0.44	0.3	0.3	1.0
rs2838961	21	*SLC19A1*	1388	0.34	1.0	0.3	1.0
rs10501325	11	*FOLH1*	1388	0.07	0.1	0.3	1.0
rs9606756	22	*TCN2*	1388	0.10	0.7	0.3	1.0
NA	1	*MTR_01_2_i*	1388	0.17	0.7	0.3	1.0
rs12591436	15	*MTHFS*	1388	0.35	0.6	0.3	1.0
rs1256095	14	*MTHFD1*	1377	0.48	0.8	0.3	1.0
rs11158540	14	*MTHFD1*	1388	0.34	0.9	0.4	1.0
rs7174668	15	*MTHFS*	1388	0.20	0.2	0.4	1.0
rs7759302	6	*GNMT*	1388	0.06	1.0	0.4	1.0
rs648372	11	*FOLH1*	1370	0.17	0.5	0.4	1.0
rs7575625	2	*DNMT3A*	1388	0.47	0.9	0.4	1.0
rs600671	15	*MTHFS*	1387	0.46	0.1	0.4	1.0
rs10932605	2	*ATIC*	1388	0.14	0.3	0.4	1.0
rs4144700	11	*FOLH1*	1388	0.38	0.3	0.4	1.0
rs1801131	1	*MTHFR*	1322	0.28	0.9	0.4	1.0
rs898436	15	*MTHFS*	1384	0.46	0.1	0.4	1.0
rs6713377	2	*DNMT3A*	1387	0.47	0.9	0.4	1.0
rs282776	15	*MTHFS*	1388	0.36	1.0	0.4	1.0
rs8101626	19	*DNMT1*	1388	0.39	0.7	0.4	1.0
rs4779141	15	*MTHFS*	1386	0.34	0.8	0.4	1.0
rs6750194	2	*ATIC*	1375	0.08	0.4	0.4	1.0
rs202718	11	*FOLH1*	1388	0.15	0.9	0.4	1.0
rs11869600	17	*PEMT*	1386	0.38	0.3	0.4	1.0
rs9001	3	*CHDH*	1385	0.10	0.3	0.4	1.0
rs4244599	17	*PEMT*	1371	0.46	0.4	0.4	1.0
rs4646340	17	*PEMT*	1388	0.36	0.5	0.4	1.0
rs4987173	6	*GNMT*	1388	0.51	0.8	0.4	1.0

Supplementary Table S5-6 (cont.) Uncorrected and corrected p-values for the interaction between D4Z4 methylation levels and selected SNPs in the one-carbon metabolism pathway in the Spanish Bladder Cancer/EPICURO Study

dbSNP ID (N=496)	Chromosome	Gene	N	MAF	*P* HWE	*P* LRT	Corrected *P* LRT
rs12614943	2	*ATIC*	1388	0.28	1.0	0.4	1.0
rs8128676	21	*SLC19A1*	1350	0.22	1.0	0.4	1.0
rs759920	19	*DNMT1*	1388	0.47	0.7	0.4	1.0
rs1076991	14	*MTHFD1*	1388	0.45	1.0	0.4	1.0
rs1109859	17	*PEMT*	1367	0.18	0.8	0.4	1.0
rs2866358	11	*FOLH1*	1372	0.38	0.2	0.4	1.0
rs4646350	17	*PEMT*	1388	0.36	0.6	0.4	1.0
rs5749131	22	*TCN2*	1388	0.43	0.2	0.4	1.0
rs2275566	1	*MTR*	1388	0.42	0.1	0.4	1.0
rs4925048	17	*PEMT*	1386	0.09	0.1	0.4	1.0
rs8019804	14	*MTHFD1*	1388	0.07	1.0	0.4	1.0
rs204942	15	*MTHFS*	1388	0.20	0.1	0.4	1.0
rs282814	15	*MTHFS*	1388	0.21	0.8	0.4	1.0
rs2124344	17	*PEMT*	1387	0.36	0.7	0.4	1.0
rs11158538	14	*MTHFD1*	1385	0.45	0.9	0.4	1.0
rs2838965	21	*SLC19A1*	1384	0.42	0.05	0.4	1.0
rs2275565	1	*MTR*	1388	0.20	0.6	0.4	1.0
rs3893384	15	*MTHFS*	1388	0.42	0.5	0.4	1.0
rs1081231	15	*MTHFS*	1387	0.18	0.8	0.4	1.0
rs2288349	19	*DNMT1*	1387	0.39	0.5	0.4	1.0
rs2983733	14	*MTHFD1*	1388	0.45	0.9	0.4	1.0
rs7951180	11	*FOLH1*	1369	0.17	0.4	0.4	1.0
rs685487	15	*MTHFS*	1388	0.37	0.09	0.4	1.0
rs1256114	14	*MTHFD1*	1387	0.11	0.7	0.4	1.0
rs2733103	15	*MTHFS*	1387	0.15	0.2	0.4	1.0
rs2983736	14	*MTHFD1*	1385	0.45	0.9	0.4	1.0
rs1956545	14	*MTHFD1*	1387	0.08	0.5	0.4	1.0
rs750546	17	*PEMT*	1373	0.43	0.5	0.4	1.0
rs10948059	6	*GNMT*	1370	0.48	0.8	0.4	1.0
rs944422	21	*SLC19A1*	1384	0.35	0.9	0.4	1.0
rs3893350	15	*MTHFS*	1388	0.13	0.1	0.4	1.0
rs1802059	5	*MTRR*	1387	0.37	0.4	0.5	1.0
rs2228611	19	*DNMT1*	1388	0.47	0.8	0.5	1.0
rs12910340	15	*MTHFS*	1388	0.42	1.0	0.5	1.0
rs12121543	1	*MTHFR*	1388	0.22	0.9	0.5	1.0
rs4531931	2	*ATIC*	1387	0.31	0.5	0.5	1.0
rs617219	5	*BHMT*	1387	0.32	0.7	0.5	1.0
rs10420338	19	*DNMT1*	1388	0.47	0.6	0.5	1.0
rs8129350	21	*SLC19A1*	1387	0.35	0.9	0.5	1.0
rs12797843	11	*FOLH1*	1388	0.13	0.6	0.5	1.0

Supplementary Table S5-6 (cont.) Uncorrected and corrected p-values for the interaction between D4Z4 methylation levels and selected SNPs in the one-carbon metabolism pathway in the Spanish Bladder Cancer/EPICURO Study

dbSNP ID (N=496)	Chromosome	Gene	N	MAF	*P* HWE	*P* LRT	Corrected *P* LRT
rs9835128	3	*CHDH*	1387	0.17	0.4	0.5	1.0
rs16971249	15	*MTHFS*	1388	0.07	0.08	0.5	1.0
rs17291414	19	*DNMT1*	1388	0.27	0.5	0.5	1.0
rs12899781	15	*MTHFS*	1383	0.17	0.06	0.5	1.0
rs10165919	2	*ATIC*	1387	0.35	0.6	0.5	1.0
rs17285431	15	*MTHFS*	1388	0.17	0.1	0.5	1.0
rs1888533	21	*SLC19A1*	1388	0.48	0.3	0.5	1.0
rs10498034	2	*ATIC*	1388	0.17	1.0	0.5	1.0
rs3800292	6	*GNMT*	1388	0.06	0.8	0.5	1.0
rs4646385	17	*PEMT*	1388	0.43	0.5	0.5	1.0
rs4817580	21	*GART*	1387	0.10	0.5	0.5	1.0
rs1369703	2	*DNMT3A*	1388	0.45	0.2	0.5	1.0
rs3740394	10	*AS3MT*	1387	0.12	0.3	0.5	1.0
rs1460177	15	*MTHFS*	1388	0.09	0.4	0.5	1.0
rs11887120	2	*DNMT3A*	1388	0.42	0.5	0.5	1.0
rs853858	20	*DNMT3B*	1386	0.37	0.2	0.5	1.0
rs11040416	11	*FOLH1*	1388	0.42	0.4	0.5	1.0
rs10509760	10	*AS3MT*	1388	0.12	0.3	0.5	1.0
rs1256112	14	*MTHFD1*	1388	0.40	0.4	0.5	1.0
rs699517	18	*TYMS*	1387	0.31	0.6	0.5	1.0
rs1059394	18	*TYMS*	1387	0.31	0.6	0.5	1.0
rs1077965	15	*MTHFS*	1387	0.41	0.4	0.5	1.0
rs372447	15	*MTHFS*	1388	0.38	0.9	0.5	1.0
rs8015278	14	*MTHFD1*	1388	0.07	0.8	0.5	1.0
rs6760069	2	*ATIC*	1387	0.14	0.5	0.5	1.0
rs17556442	11	*FOLH1*	1384	0.06	0.05	0.5	1.0
rs748196	17	*PEMT*	1385	0.42	0.9	0.5	1.0
rs443394	15	*MTHFS*	1388	0.41	0.3	0.5	1.0
rs8011839	14	*MTHFD1*	1388	0.18	0.4	0.5	1.0
rs2289195	2	*DNMT3A*	1387	0.44	0.2	0.5	1.0
rs11627525	14	*MTHFD1*	1388	0.10	0.3	0.5	1.0
rs7604984	2	*ATIC*	1388	0.40	0.4	0.5	1.0
rs7594432	2	*DNMT3A*	1388	0.45	0.3	0.5	1.0
rs10498036	2	*ATIC*	1388	0.40	0.4	0.5	1.0
rs11871738	17	*PEMT*	1388	0.38	0.4	0.6	1.0
rs7177659	15	*MTHFS*	1387	0.50	0.08	0.6	1.0
rs9621049	22	*TCN2*	1388	0.10	0.8	0.6	1.0
rs378057	15	*MTHFS*	1387	0.14	1.0	0.6	1.0
rs202712	11	*FOLH1*	1387	0.23	0.7	0.6	1.0
rs7177027	15	*MTHFS*	1388	0.24	0.08	0.6	1.0

Supplementary Table S5-6 (cont.) Uncorrected and corrected p-values for the interaction between D4Z4 methylation levels and selected SNPs in the one-carbon metabolism pathway in the Spanish Bladder Cancer/EPICURO Study

dbSNP ID (N=496)	Chromosome	Gene	N	MAF	*P* HWE	*P* LRT	Corrected *P* LRT
rs7604425	2	*ATIC*	1388	0.35	0.7	0.6	1.0
rs6511677	19	*DNMT1*	1387	0.39	0.6	0.6	1.0
rs6722613	2	*DNMT3A*	1388	0.40	1.0	0.6	1.0
rs8034036	15	*MTHFS*	1386	0.11	0.09	0.6	1.0
rs11191439	10	*AS3MT*	1387	0.12	0.2	0.6	1.0
rs11694842	2	*DNMT3A*	1387	0.28	0.4	0.6	1.0
rs2162560	19	*DNMT1*	1387	0.39	0.6	0.6	1.0
rs10839239	11	*FOLH1*	1386	0.23	0.6	0.6	1.0
rs4673965	2	*ATIC*	1388	0.40	1.0	0.6	1.0
rs17211644	15	*MTHFS*	1388	0.10	0.7	0.6	1.0
rs445263	15	*MTHFS*	1388	0.30	0.9	0.6	1.0
rs11687225	2	*ATIC*	1387	0.40	0.9	0.6	1.0
rs12997662	2	*ATIC*	1387	0.33	0.7	0.6	1.0
rs16853834	2	*ATIC*	1388	0.16	1.0	0.6	1.0
rs660439	11	*FOLH1*	1384	0.23	0.7	0.6	1.0
rs734693	2	*DNMT3A*	1387	0.29	0.2	0.6	1.0
rs4902278	14	*MTHFD1*	1383	0.07	0.6	0.6	1.0
rs4646383	17	*PEMT*	1387	0.09	0.5	0.6	1.0
rs8923	15	*MTHFS*	1388	0.08	0.1	0.6	1.0
rs8128050	21	*SLC19A1*	1385	0.35	0.9	0.6	1.0
rs4820887	22	*TCN2*	1388	0.09	0.6	0.6	1.0
rs11892646	2	*DNMT3A*	1387	0.12	0.6	0.6	1.0
rs2409495	21	*GART*	1388	0.19	0.05	0.6	1.0
rs3783	17	*SHMT1*	1398	0.26	0.6	0.6	1.0
rs1464864	2	*ATIC*	1388	0.30	0.8	0.6	1.0
rs4369857	2	*ATIC*	1388	0.05	0.7	0.6	1.0
rs17824591	14	*MTHFD1*	1386	0.23	0.2	0.6	1.0
rs16999714	19	*DNMT1*	1386	0.21	0.1	0.6	1.0
rs8068641	17	*PEMT*	1382	0.11	0.4	0.6	1.0
rs1801394	5	*MTRR*	1398	0.49	0.8	0.6	1.0
rs1081235	15	*MTHFS*	1388	0.19	0.3	0.6	1.0
rs2987969	14	*MTHFD1*	1387	0.45	0.7	0.6	1.0
rs17728676	11	*FOLH1*	1387	0.07	0.1	0.6	1.0
rs234706	21	*CBS*	1388	0.32	0.1	0.6	1.0
rs166868	15	*MTHFS*	1387	0.36	1.0	0.6	1.0
rs2289209	3	*CHDH*	1388	0.05	1.0	0.6	1.0
rs731991	22	*TCN2*	1343	0.48	0.1	0.7	1.0
NA	1	*MTHFR_02_2_i*	1388	0.40	0.5	0.7	1.0
rs1127717	3	*ALDH1L1*	1388	0.24	0.6	0.7	1.0
rs1801133	1	*MTHFR*	1388	0.40	0.5	0.7	1.0

Supplementary Table S5-6 (cont.) Uncorrected and corrected p-values for the interaction between D4Z4 methylation levels and selected SNPs in the one-carbon metabolism pathway in the Spanish Bladder Cancer/EPICURO Study

dbSNP ID (N=496)	Chromosome	Gene	N	MAF	*P* HWE	*P* LRT	Corrected *P* LRT
rs11855092	15	*MTHFS*	1388	0.23	0.07	0.7	1.0
rs7144437	14	*MTHFD1*	1387	0.07	0.6	0.7	1.0
rs2288350	19	*DNMT1*	1387	0.08	0.6	0.7	1.0
rs12898670	15	*MTHFS*	1387	0.32	0.9	0.7	1.0
rs4672768	2	*ATIC*	1385	0.31	0.9	0.7	1.0
rs2267163	22	*TCN2*	1386	0.43	0.2	0.7	1.0
rs1055345	21	*SLC19A1*	1387	0.29	0.3	0.7	1.0
rs4673993	2	*ATIC*	1388	0.31	0.9	0.7	1.0
rs8018032	14	*MTHFD1*	1388	0.45	0.7	0.7	1.0
rs12797853	11	*FOLH1*	1385	0.13	0.7	0.7	1.0
rs1801198	22	*TCN2*	1387	0.43	0.3	0.7	1.0
rs8081810	17	*PEMT*	1387	0.21	0.9	0.7	1.0
rs1979276	17	*SHMT1*	1395	0.30	1.0	0.7	1.0
rs8111085	19	*DNMT1*	1387	0.08	0.6	0.7	1.0
rs11658944	17	*PEMT*	1387	0.05	0.7	0.7	1.0
rs914238	21	*SLC19A1*	1388	0.49	0.4	0.7	1.0
rs2289093	2	*DNMT3A*	1388	0.28	0.2	0.7	1.0
rs10839295	11	*FOLH1*	1387	0.39	0.5	0.7	1.0
rs2150460	21	*SLC19A1*	1388	0.21	0.9	0.7	1.0
rs2790	18	*TYMS*	1384	0.20	0.7	0.7	1.0
rs11681447	2	*DNMT3A*	1386	0.28	0.2	0.7	1.0
rs4673991	2	*ATIC*	1387	0.31	0.9	0.7	1.0
rs4610054	2	*ATIC*	1383	0.38	1.0	0.7	1.0
rs282772	15	*MTHFS*	1388	0.13	0.3	0.7	1.0
rs1650697	5	*DHFR*	1385	0.23	0.1	0.7	1.0
rs8003379	14	*MTHFD1*	1387	0.24	0.4	0.7	1.0
rs11683424	2	*DNMT3A*	1388	0.12	0.5	0.7	1.0
rs17284990	15	*MTHFS*	1388	0.22	0.5	0.7	1.0
rs11672909	19	*DNMT1*	1387	0.08	0.6	0.7	1.0
rs2838969	21	*SLC19A1*	1388	0.07	1.0	0.7	1.0
rs2183601	21	*SLC19A1*	1388	0.21	1.0	0.7	1.0
rs6801605	3	*CHDH*	1388	0.38	0.6	0.7	1.0
rs9306139	21	*SLC19A1*	1386	0.21	0.8	0.7	1.0
rs8003567	14	*MTHFD1*	1388	0.10	0.8	0.7	1.0
rs10460566	2	*DNMT3A*	1388	0.27	0.3	0.7	1.0
rs2838973	21	*SLC19A1*	1388	0.21	1.0	0.7	1.0
rs7581217	2	*DNMT3A*	1388	0.39	0.2	0.7	1.0
rs9976878	21	*SLC19A1*	1387	0.21	0.8	0.7	1.0
rs282792	15	*MTHFS*	1388	0.39	0.6	0.7	1.0
rs1814175	11	*FOLH1*	1384	0.39	0.4	0.7	1.0

Supplementary Table S5-6 (cont.) Uncorrected and corrected p-values for the interaction between D4Z4 methylation levels and selected SNPs in the one-carbon metabolism pathway in the Spanish Bladder Cancer/EPICURO Study

dbSNP ID (N=496)	Chromosome	Gene	N	MAF	*P* HWE	*P* LRT	Corrected *P* LRT
rs10418707	19	*DNMT1*	1387	0.08	0.6	0.8	1.0
rs749130	2	*DNMT3A*	1388	0.44	0.6	0.8	1.0
rs9789571	2	*ATIC*	1388	0.42	1.0	0.8	1.0
rs3862350	11	*FOLH1*	1360	0.39	0.2	0.8	1.0
rs1020697	17	*PEMT*	1361	0.10	0.3	0.8	1.0
rs4804494	19	*DNMT1*	1387	0.08	0.6	0.8	1.0
rs9836592	3	*CHDH*	1388	0.31	0.3	0.8	1.0
rs1979277	17	*SHMT1*	1398	0.27	0.8	0.8	1.0
rs4804490	19	*DNMT1*	1386	0.08	0.6	0.8	1.0
rs11040106	11	*FOLH1*	1379	0.36	0.6	0.8	1.0
rs567754	5	*BHMT*	1387	0.29	0.9	0.8	1.0
rs11656215	17	*PEMT*	1388	0.45	0.4	0.8	1.0
rs435689	15	*MTHFS*	1388	0.49	0.9	0.8	1.0
rs4434082	21	*SLC19A1*	1387	0.21	0.9	0.8	1.0
rs1550117	2	*DNMT3A*	1387	0.08	1.0	0.8	1.0
rs2838970	21	*SLC19A1*	1387	0.39	0.9	0.8	1.0
rs376863	15	*MTHFS*	1366	0.50	0.9	0.8	1.0
rs8129445	21	*SLC19A1*	1388	0.32	0.9	0.8	1.0
rs3774616	3	*CHDH*	1388	0.05	1.0	0.8	1.0
rs3818239	14	*MTHFD1*	1380	0.13	0.9	0.8	1.0
rs6058896	20	*DNMT3B*	1388	0.08	0.8	0.8	1.0
rs1808119	2	*ATIC*	1388	0.20	1.0	0.8	1.0
rs8128681	21	*SLC19A1*	1388	0.32	0.9	0.8	1.0
rs9462856	6	*GNMT*	1388	0.41	0.9	0.8	1.0
rs1809986	11	*FOLH1*	1388	0.37	1.0	0.8	1.0
rs1917321	11	*FOLH1*	1380	0.50	0.2	0.8	1.0
rs7085104	10	*AS3MT*	1388	0.39	1.0	0.8	1.0
rs1023159	21	*SLC19A1*	1386	0.42	0.4	0.8	1.0
rs7279305	21	*SLC19A1*	1388	0.35	1.0	0.8	1.0
rs7120743	11	*FOLH1*	1371	0.37	0.8	0.8	1.0
rs13427202	2	*DNMT3A*	1387	0.47	0.6	0.8	1.0
rs6058894	20	*DNMT3B*	1387	0.08	0.5	0.8	1.0
rs4673981	2	*ATIC*	1388	0.39	0.8	0.8	1.0
rs2116940	19	*DNMT1*	1387	0.08	0.6	0.8	1.0
rs12987326	2	*DNMT3A*	1388	0.37	0.7	0.8	1.0
rs4778721	15	*MTHFS*	1388	0.23	0.2	0.8	1.0
rs9977111	21	*SLC19A1*	1355	0.33	0.07	0.8	1.0
rs4804125	19	*DNMT1*	1387	0.08	0.6	0.8	1.0
rs1473406	15	*MTHFS*	1386	0.15	0.9	0.8	1.0
rs4778719	15	*MTHFS*	1388	0.23	0.2	0.8	1.0

Supplementary Table S5-6 (cont.) Uncorrected and corrected p-values for the interaction between D4Z4 methylation levels and selected SNPs in the one-carbon metabolism pathway in the Spanish Bladder Cancer/EPICURO Study

dbSNP ID (N=496)	Chromosome	Gene	N	MAF	*P* HWE	*P* LRT	Corrected *P* LRT
rs202700	11	*FOLH1*	1337	0.23	0.8	0.8	1.0
rs6586282	21	*CBS*	1387	0.17	0.2	0.8	1.0
rs4393531	15	*MTHFS*	1386	0.48	0.4	0.8	1.0
rs1465825	2	*DNMT3A*	1387	0.27	0.3	0.8	1.0
rs9305012	19	*DNMT1*	1387	0.08	0.6	0.8	1.0
rs17751556	14	*MTHFD1*	1388	0.08	1.0	0.8	1.0
rs6445606	3	*CHDH*	1388	0.29	0.2	0.9	1.0
rs2301955	22	*TCN2*	1386	0.43	0.3	0.9	1.0
rs12462004	19	*DNMT1*	1386	0.08	0.6	0.9	1.0
rs2838977	21	*SLC19A1*	1386	0.39	0.9	0.9	1.0
rs8112895	19	*DNMT1*	1388	0.08	0.6	0.9	1.0
rs9621047	22	*TCN2*	1388	0.44	0.2	0.9	1.0
rs2586182	15	*MTHFS*	1388	0.15	0.4	0.9	1.0
rs17209637	15	*MTHFS*	1384	0.25	1.0	0.9	1.0
rs767138	21	*SLC19A1*	1385	0.40	0.6	0.9	1.0
rs13002567	2	*DNMT3A*	1388	0.28	0.5	0.9	1.0
rs5753231	22	*TCN2*	1387	0.16	0.8	0.9	1.0
rs4476347	2	*ATIC*	1387	0.25	0.4	0.9	1.0
rs7586294	2	*DNMT3A*	1387	0.47	0.5	0.9	1.0
rs5749135	22	*TCN2*	1388	0.43	0.3	0.9	1.0
rs4779148	15	*MTHFS*	1388	0.11	0.2	0.9	1.0
rs16971252	15	*MTHFS*	1388	0.06	0.3	0.9	1.0
rs2877078	21	*SLC19A1*	1376	0.39	0.7	0.9	1.0
rs2241807	3	*CHDH*	1388	0.43	0.7	0.9	1.0
rs437302	20	*DNMT3B*	1388	0.09	1.0	0.9	1.0
rs897453	17	*PEMT*	1384	0.49	0.6	0.9	1.0
rs2586154	15	*MTHFS*	1388	0.15	0.5	0.9	1.0
rs11629135	14	*MTHFD1*	1388	0.10	0.7	0.9	1.0
rs2586153	15	*MTHFS*	1374	0.15	0.4	0.9	1.0
rs11701960	21	*SLC19A1*	1387	0.17	1.0	0.9	1.0
rs1604503	15	*MTHFS*	1388	0.14	0.4	0.9	1.0
NA	1	*MTHFR_02_OR*	1285	0.39	0.4	0.9	1.0
rs6518253	21	*SLC19A1*	1387	0.46	0.3	0.9	1.0
rs12148881	15	*MTHFS*	1387	0.27	0.2	0.9	1.0
rs4820888	22	*TCN2*	1387	0.47	0.1	0.9	1.0
rs2424908	20	*DNMT3B*	1388	0.17	0.7	0.9	1.0
rs7276295	21	*SLC19A1*	1388	0.06	0.3	0.9	1.0
rs7219568	17	*PEMT*	1388	0.05	1.0	0.9	1.0
rs2987981	14	*MTHFD1*	1388	0.25	0.9	0.9	1.0

Supplementary Table S5-6 (cont.) Uncorrected and corrected p-values for the interaction between D4Z4 methylation levels and selected SNPs in the one-carbon metabolism pathway in the Spanish Bladder Cancer/EPICURO Study

dbSNP ID (N=496)	Chromosome	Gene	N	MAF	*P* HWE	*P* LRT	Corrected *P* LRT
rs582172	15	*MTHFS*	1388	0.41	0.6	0.9	1.0
rs3785499	17	*PEMT*	1388	0.47	0.8	0.9	1.0
rs2236222	14	*MTHFD1*	1388	0.10	0.5	0.9	1.0
rs12627639	21	*SLC19A1*	1387	0.21	0.5	0.9	1.0
rs8971	21	*GART*	1386	0.26	0.8	0.9	1.0
rs16971231	15	*MTHFS*	1385	0.05	0.1	0.9	1.0
rs4924922	17	*PEMT*	1388	0.36	0.3	0.9	1.0
rs13036246	2	*DNMT3A*	1387	0.49	0.4	1.0	1.0
rs3783728	14	*MTHFD1*	1388	0.08	1.0	1.0	1.0
rs6485991	11	*FOLH1*	1382	0.16	0.6	1.0	1.0
rs12453139	17	*PEMT*	1386	0.26	0.9	1.0	1.0
rs4819138	21	*SLC19A1*	1388	0.39	0.8	1.0	1.0
rs2733106	15	*MTHFS*	1383	0.15	0.5	1.0	1.0
rs2834231	21	*GART*	1388	0.26	0.7	1.0	1.0
rs1404774	2	*ATIC*	1380	0.21	0.4	1.0	1.0
rs17279753	15	*MTHFS*	1388	0.20	0.5	1.0	1.0
rs2834232	21	*GART*	1387	0.26	0.7	1.0	1.0
rs17279885	15	*MTHFS*	1388	0.21	0.7	1.0	1.0
rs12373907	21	*SLC19A1*	1387	0.38	0.4	1.0	1.0

Supplementary Table S5-6 (cont.) Uncorrected and corrected p-values for the interaction between D4Z4 methylation levels and selected SNPs in the one-carbon metabolism pathway in the Spanish Bladder Cancer/EPICURO Study

SNPs modeled in codominant mode of inheritance							
dbSNP ID (N=375)	Chromosome	Gene	N	MAF	*P* HWE	*P* LRT	Corrected *P* LRT
rs1256142	14	*MTHFD1*	1388	0.44	0.07	0.002	0.4
rs2838965	21	*SLC19A1*	1384	0.42	0.05	0.004	0.6
rs7124266	11	*FOLH1*	1388	0.30	0.6	0.01	0.9
rs2236224	14	*MTHFD1*	1388	0.36	0.1	0.01	0.9
rs8041943	15	*MTHFS*	1385	0.42	0.6	0.01	0.9
rs2834235	21	*GART*	1388	0.38	0.7	0.01	0.9
rs3821353	2	*ATIC*	1388	0.21	0.4	0.02	1.0
rs6087990	20	*DNMT3B*	1388	0.33	0.6	0.02	1.0
rs2865908	11	*FOLH1*	1388	0.19	0.9	0.02	1.0
rs4094478	11	*FOLH1*	1365	0.20	0.7	0.02	1.0
rs2236225	14	*MTHFD1*	1387	0.43	0.2	0.02	1.0
rs10839210	11	*FOLH1*	1386	0.21	0.5	0.02	1.0
rs3893384	15	*MTHFS*	1388	0.42	0.5	0.02	1.0
rs3862342	11	*FOLH1*	1386	0.28	0.8	0.02	1.0
rs10769558	11	*FOLH1*	1388	0.21	0.5	0.02	1.0
rs16853782	2	*ATIC*	1388	0.21	0.7	0.03	1.0
rs1847638	11	*FOLH1*	1335	0.21	1.0	0.03	1.0
rs3772078	2	*ATIC*	1388	0.21	0.6	0.03	1.0
rs11158542	14	*MTHFD1*	1388	0.29	0.8	0.03	1.0
rs2305230	3	*ALDH1L1*	1387	0.19	0.7	0.03	1.0
rs6058869	20	*DNMT3B*	1388	0.33	0.7	0.04	1.0
rs2154583	21	*GART*	1384	0.39	0.7	0.04	1.0
rs11191457	10	*AS3MT*	1385	0.23	0.4	0.04	1.0
rs6517178	21	*GART*	1388	0.39	0.7	0.04	1.0
rs4441015	11	*FOLH1*	1353	0.15	0.5	0.04	1.0
rs282792	15	*MTHFS*	1388	0.39	0.6	0.04	1.0
rs6573559	14	*MTHFD1*	1388	0.29	1.0	0.04	1.0
rs11040198	11	*FOLH1*	1381	0.21	0.2	0.05	1.0
rs12910340	15	*MTHFS*	1388	0.42	1.0	0.05	1.0
rs2696923	11	*FOLH1*	1388	0.21	0.3	0.05	1.0
rs7085854	10	*AS3MT*	1387	0.23	0.6	0.05	1.0
rs6058893	20	*DNMT3B*	1388	0.33	0.2	0.05	1.0
rs7111711	11	*FOLH1*	1388	0.25	0.9	0.05	1.0
rs7107178	11	*FOLH1*	1387	0.25	1.0	0.06	1.0
rs10839296	11	*FOLH1*	1371	0.25	0.8	0.06	1.0
rs2696935	11	*FOLH1*	1388	0.21	0.4	0.06	1.0
rs4817579	21	*GART*	1388	0.35	1.0	0.07	1.0
rs1081235	15	*MTHFS*	1388	0.19	0.3	0.07	1.0

Supplementary Table S5-6 (cont.) Uncorrected and corrected p-values for the interaction between D4Z4 methylation levels and selected SNPs in the one-carbon metabolism pathway in the Spanish Bladder Cancer/EPICURO Study

dbSNP ID (N=375)	Chromosome	Gene	N	MAF	*P* HWE	*P* LRT	Corrected *P* LRT
rs4817577	21	*GART*	1388	0.34	0.9	0.07	1.0
rs11627387	14	*MTHFD1*	1388	0.29	0.9	0.07	1.0
rs4911107	20	*DNMT3B*	1388	0.32	0.6	0.08	1.0
rs7283354	21	*GART*	1388	0.34	0.9	0.08	1.0
rs2035027	15	*MTHFS*	1388	0.16	0.5	0.08	1.0
rs11040421	11	*FOLH1*	1388	0.15	0.6	0.08	1.0
rs6495441	15	*MTHFS*	1388	0.24	0.3	0.09	1.0
rs4779140	15	*MTHFS*	1387	0.48	0.6	0.09	1.0
rs6058897	20	*DNMT3B*	1388	0.43	0.2	0.09	1.0
rs2281603	14	*MTHFD1*	1388	0.19	0.2	0.1	1.0
rs16999714	19	*DNMT1*	1386	0.21	0.1	0.1	1.0
rs12438477	15	*MTHFS*	1387	0.36	0.4	0.1	1.0
rs473334	1	*CTH*	1388	0.31	0.9	0.1	1.0
rs1880580	15	*MTHFS*	1388	0.30	0.09	0.1	1.0
rs4779165	15	*MTHFS*	1387	0.16	0.4	0.1	1.0
rs2838958	21	*SLC19A1*	1385	0.45	0.7	0.1	1.0
rs372447	15	*MTHFS*	1388	0.38	0.9	0.1	1.0
rs663649	1	*CTH*	1388	0.31	0.9	0.1	1.0
rs865646	5	*DHFR*	1340	0.34	0.4	0.1	1.0
rs3785499	17	*PEMT*	1388	0.47	0.8	0.1	1.0
rs679470	11	*FOLH1*	1387	0.17	0.6	0.1	1.0
rs7563206	2	*ATIC*	1387	0.47	0.6	0.1	1.0
rs16971450	15	*MTHFS*	1387	0.16	0.4	0.1	1.0
rs9974061	21	*SLC19A1*	1388	0.18	1.0	0.1	1.0
rs648372	11	*FOLH1*	1370	0.17	0.5	0.1	1.0
rs9835128	3	*CHDH*	1387	0.17	0.4	0.1	1.0
rs7560488	2	*DNMT3A*	1333	0.47	0.2	0.1	1.0
rs1880586	2	*ATIC*	1387	0.47	0.6	0.1	1.0
rs2424932	20	*DNMT3B*	1387	0.43	0.5	0.1	1.0
rs2424928	20	*DNMT3B*	1388	0.39	0.2	0.1	1.0
rs4646404	17	*PEMT*	1383	0.36	0.3	0.1	1.0
rs585800	5	*BHMT*	1388	0.26	0.05	0.1	1.0
rs7951180	11	*FOLH1*	1369	0.17	0.4	0.2	1.0
rs9323450	14	*MTHFD1*	1388	0.30	0.8	0.2	1.0
rs2424921	20	*DNMT3B*	1388	0.39	0.3	0.2	1.0
rs3783	17	*SHMT1*	1398	0.26	0.6	0.2	1.0
rs4911263	20	*DNMT3B*	1388	0.32	1.0	0.2	1.0
rs202676	11	*FOLH1*	1380	0.17	0.8	0.2	1.0
rs2586154	15	*MTHFS*	1388	0.15	0.5	0.2	1.0
rs6141813	20	*DNMT3B*	1388	0.13	0.8	0.2	1.0

Supplementary Table S5-6 (cont.) Uncorrected and corrected p-values for the interaction between D4Z4 methylation levels and selected SNPs in the one-carbon metabolism pathway in the Spanish Bladder Cancer/EPICURO Study

dbSNP ID (N=375)	Chromosome	Gene	N	MAF	*P* HWE	*P* LRT	Corrected *P* LRT
rs7587636	2	*DNMT3A*	1388	0.45	0.1	0.2	1.0
rs12903985	15	*MTHFS*	1388	0.28	0.4	0.2	1.0
rs6058891	20	*DNMT3B*	1386	0.39	0.2	0.2	1.0
rs2424922	20	*DNMT3B*	1387	0.39	0.2	0.2	1.0
rs9332	5	*MTRR*	1388	0.12	0.2	0.2	1.0
rs7605753	2	*DNMT3A*	1388	0.47	0.6	0.2	1.0
rs6087988	20	*DNMT3B*	1388	0.20	0.6	0.2	1.0
rs6749992	2	*DNMT3A*	1388	0.47	0.8	0.2	1.0
rs559062	1	*CTH*	1388	0.22	0.6	0.2	1.0
rs12898642	15	*MTHFS*	1387	0.43	0.2	0.2	1.0
rs7111215	11	*FOLH1*	1368	0.40	0.2	0.2	1.0
rs13401241	2	*DNMT3A*	1388	0.45	0.1	0.2	1.0
rs7220132	17	*PEMT*	1388	0.29	0.7	0.2	1.0
rs10418	22	*TCN2*	1367	0.21	0.7	0.2	1.0
rs1917311	11	*FOLH1*	1353	0.40	0.4	0.2	1.0
rs1801131	1	*MTHFR*	1322	0.28	0.9	0.2	1.0
rs7583409	2	*DNMT3A*	1387	0.35	0.4	0.2	1.0
rs10163099	15	*MTHFS*	1385	0.26	0.1	0.2	1.0
rs9306139	21	*SLC19A1*	1386	0.21	0.8	0.2	1.0
rs2586182	15	*MTHFS*	1388	0.15	0.4	0.2	1.0
rs910085	20	*DNMT3B*	1387	0.29	0.5	0.2	1.0
rs2424914	20	*DNMT3B*	1388	0.39	0.4	0.2	1.0
rs4479310	17	*PEMT*	1388	0.29	0.6	0.2	1.0
rs1164685	11	*FOLH1*	1385	0.38	0.3	0.2	1.0
rs6058883	20	*DNMT3B*	1387	0.39	0.3	0.2	1.0
rs6495446	15	*MTHFS*	1387	0.26	0.1	0.2	1.0
rs9976878	21	*SLC19A1*	1387	0.21	0.8	0.2	1.0
rs663465	1	*CTH*	1387	0.41	0.4	0.2	1.0
rs4987173	6	*GNMT*	1388	0.51	0.8	0.2	1.0
rs2150460	21	*SLC19A1*	1388	0.21	0.9	0.2	1.0
rs2183601	21	*SLC19A1*	1388	0.21	1.0	0.2	1.0
rs1074390	15	*MTHFS*	1388	0.37	0.6	0.3	1.0
rs2838973	21	*SLC19A1*	1388	0.21	1.0	0.3	1.0
rs8074074	17	*PEMT*	1386	0.29	0.8	0.3	1.0
rs4818789	21	*SLC19A1*	1388	0.25	0.5	0.3	1.0
rs10420338	19	*DNMT1*	1388	0.47	0.6	0.3	1.0
rs4646410	17	*PEMT*	1384	0.31	0.9	0.3	1.0
rs2733103	15	*MTHFS*	1387	0.15	0.2	0.3	1.0
rs8659	5	*MTRR*	1386	0.35	0.3	0.3	1.0
rs4434082	21	*SLC19A1*	1387	0.21	0.9	0.3	1.0

Supplementary Table S5-6 (cont.) Uncorrected and corrected p-values for the interaction between D4Z4 methylation levels and selected SNPs in the one-carbon metabolism pathway in the Spanish Bladder Cancer/EPICURO Study

dbSNP ID (N=375)	Chromosome	Gene	N	MAF	*P* HWE	*P* LRT	Corrected *P* LRT
rs914238	21	*SLC19A1*	1388	0.49	0.4	0.3	1.0
rs7946	17	*PEMT*	1387	0.29	0.4	0.3	1.0
rs759920	19	*DNMT1*	1388	0.47	0.7	0.3	1.0
rs9890064	17	*PEMT*	1388	0.43	0.4	0.3	1.0
rs2424913	20	*DNMT3B*	1387	0.37	0.2	0.3	1.0
rs2424906	20	*DNMT3B*	1388	0.38	0.2	0.3	1.0
rs12373907	21	*SLC19A1*	1387	0.38	0.4	0.3	1.0
rs1801133	1	*MTHFR*	1388	0.40	0.5	0.3	1.0
rs2586153	15	*MTHFS*	1374	0.15	0.4	0.3	1.0
rs2838956	21	*SLC19A1*	1373	0.44	0.07	0.3	1.0
rs2228611	19	*DNMT1*	1388	0.47	0.8	0.3	1.0
NA	1	*MTHFR_02_2_i*	1388	0.40	0.5	0.3	1.0
rs6706415	2	*ATIC*	1388	0.30	0.4	0.3	1.0
rs4819128	21	*SLC19A1*	1388	0.44	0.2	0.3	1.0
rs11085720	19	*DNMT1*	1388	0.42	0.5	0.3	1.0
rs1846285	11	*FOLH1*	1385	0.17	0.2	0.3	1.0
rs7581217	2	*DNMT3A*	1388	0.39	0.2	0.3	1.0
rs1051266	21	*SLC19A1*	1388	0.45	0.2	0.3	1.0
rs435689	15	*MTHFS*	1388	0.49	0.9	0.3	1.0
rs4144700	11	*FOLH1*	1388	0.38	0.3	0.3	1.0
rs3788200	21	*SLC19A1*	1388	0.45	0.3	0.3	1.0
rs4924922	17	*PEMT*	1388	0.36	0.3	0.3	1.0
rs588458	11	*FOLH1*	1367	0.38	0.3	0.3	1.0
rs4672768	2	*ATIC*	1385	0.31	0.9	0.3	1.0
rs3760188	17	*PEMT*	1388	0.46	0.6	0.3	1.0
rs2733106	15	*MTHFS*	1383	0.15	0.5	0.3	1.0
rs12614943	2	*ATIC*	1388	0.28	1.0	0.3	1.0
rs1046778	10	*AS3MT*	1388	0.33	0.8	0.3	1.0
rs10948059	6	*GNMT*	1370	0.48	0.8	0.3	1.0
rs2866358	11	*FOLH1*	1372	0.38	0.2	0.3	1.0
rs7174668	15	*MTHFS*	1388	0.20	0.2	0.3	1.0
rs2290684	19	*DNMT1*	1388	0.47	0.8	0.3	1.0
rs2115536	15	*MTHFS*	1388	0.50	0.05	0.4	1.0
rs3755817	3	*CHDH*	1387	0.30	0.5	0.4	1.0
rs4646344	17	*PEMT*	1388	0.47	0.7	0.4	1.0
rs10498036	2	*ATIC*	1388	0.40	0.4	0.4	1.0
rs1802059	5	*MTRR*	1387	0.37	0.4	0.4	1.0
rs8074191	17	*PEMT*	1372	0.28	0.9	0.4	1.0
rs11683424	2	*DNMT3A*	1388	0.12	0.5	0.4	1.0
rs914232	21	*SLC19A1*	1387	0.44	0.2	0.4	1.0

Supplementary Table S5-6 (cont.) Uncorrected and corrected p-values for the interaction between D4Z4 methylation levels and selected SNPs in the one-carbon metabolism pathway in the Spanish Bladder Cancer/EPICURO Study

dbSNP ID (N=375)	Chromosome	Gene	N	MAF	*P* HWE	*P* LRT	Corrected *P* LRT
rs1380642	15	*MTHFS*	1388	0.18	0.3	0.4	1.0
rs914231	21	*SLC19A1*	1383	0.44	0.2	0.4	1.0
rs1127717	3	*ALDH1L1*	1388	0.24	0.6	0.4	1.0
rs4673993	2	*ATIC*	1388	0.31	0.9	0.4	1.0
rs282795	15	*MTHFS*	1387	0.32	0.7	0.4	1.0
rs4673965	2	*ATIC*	1388	0.40	1.0	0.4	1.0
rs7604984	2	*ATIC*	1388	0.40	0.4	0.4	1.0
rs2267163	22	*TCN2*	1386	0.43	0.2	0.4	1.0
rs204942	15	*MTHFS*	1388	0.20	0.1	0.4	1.0
rs2409495	21	*GART*	1388	0.19	0.05	0.4	1.0
rs897453	17	*PEMT*	1384	0.49	0.6	0.4	1.0
rs4911108	20	*DNMT3B*	1384	0.29	0.7	0.4	1.0
rs10519256	15	*MTHFS*	1388	0.11	0.1	0.4	1.0
rs1979277	17	*SHMT1*	1398	0.27	0.8	0.4	1.0
rs1888530	21	*SLC19A1*	1358	0.47	0.07	0.4	1.0
NA	1	*MTR_01_OR*	1389	0.16	0.7	0.4	1.0
rs11677670	2	*DNMT3A*	1382	0.17	0.5	0.4	1.0
rs4673991	2	*ATIC*	1387	0.31	0.9	0.4	1.0
rs1801198	22	*TCN2*	1387	0.43	0.3	0.4	1.0
rs4646385	17	*PEMT*	1388	0.43	0.5	0.4	1.0
rs10179873	2	*ATIC*	1388	0.30	0.4	0.4	1.0
rs10460566	2	*DNMT3A*	1388	0.27	0.3	0.4	1.0
rs770144	15	*MTHFS*	1388	0.20	0.3	0.4	1.0
rs2115540	15	*MTHFS*	1387	0.50	0.05	0.4	1.0
rs1983462	2	*ATIC*	1388	0.30	0.3	0.4	1.0
rs12148881	15	*MTHFS*	1387	0.27	0.2	0.4	1.0
rs7215833	17	*PEMT*	1388	0.35	0.5	0.4	1.0
rs7177027	15	*MTHFS*	1388	0.24	0.08	0.4	1.0
rs10197653	2	*ATIC*	1388	0.29	0.1	0.4	1.0
rs1051298	21	*SLC19A1*	1387	0.46	0.3	0.4	1.0
rs1979276	17	*SHMT1*	1395	0.30	1.0	0.4	1.0
rs4646341	17	*PEMT*	1385	0.36	0.5	0.4	1.0
rs8971	21	*GART*	1386	0.26	0.8	0.4	1.0
rs7177659	15	*MTHFS*	1387	0.50	0.08	0.4	1.0
rs6722613	2	*DNMT3A*	1388	0.40	1.0	0.4	1.0
rs11871738	17	*PEMT*	1388	0.38	0.4	0.4	1.0
rs17291414	19	*DNMT1*	1388	0.27	0.5	0.4	1.0
rs17209637	15	*MTHFS*	1384	0.25	1.0	0.4	1.0
rs4819130	21	*SLC19A1*	1384	0.45	0.2	0.4	1.0
rs10197559	2	*ATIC*	1380	0.29	0.9	0.5	1.0

Supplementary Table S5-6 (cont.) Uncorrected and corrected p-values for the interaction between D4Z4 methylation levels and selected SNPs in the one-carbon metabolism pathway in the Spanish Bladder Cancer/EPICURO Study

dbSNP ID (N=375)	Chromosome	Gene	N	MAF	*P* HWE	*P* LRT	Corrected *P* LRT
rs11158540	14	*MTHFD1*	1388	0.34	0.9	0.5	1.0
rs1950902	14	*MTHFD1*	1388	0.14	0.4	0.5	1.0
rs3177999	21	*SLC19A1*	1380	0.45	0.2	0.5	1.0
rs2330183	21	*SLC19A1*	1358	0.44	0.05	0.5	1.0
rs2838961	21	*SLC19A1*	1388	0.34	1.0	0.5	1.0
rs2834231	21	*GART*	1388	0.26	0.7	0.5	1.0
rs12416687	10	*AS3MT*	1388	0.28	0.1	0.5	1.0
rs2834232	21	*GART*	1387	0.26	0.7	0.5	1.0
rs2424908	20	*DNMT3B*	1388	0.17	0.7	0.5	1.0
rs11892429	2	*ATIC*	1388	0.29	0.7	0.5	1.0
rs6711622	2	*DNMT3A*	1388	0.44	1.0	0.5	1.0
rs3788190	21	*SLC19A1*	1387	0.47	0.1	0.5	1.0
rs740234	22	*TCN2*	1388	0.23	0.8	0.5	1.0
rs515064	1	*CTH*	1388	0.36	0.8	0.5	1.0
rs4646359	17	*PEMT*	1388	0.45	0.3	0.5	1.0
rs17745484	2	*DNMT3A*	1387	0.35	0.8	0.5	1.0
rs1465825	2	*DNMT3A*	1387	0.27	0.3	0.5	1.0
rs35020344	14	*MTHFD1*	1387	0.47	0.7	0.5	1.0
NA	1	*MTHFR_02_OR*	1285	0.39	0.4	0.5	1.0
rs1256107	14	*MTHFD1*	1387	0.48	0.7	0.5	1.0
rs1805087	1	*MTR*	1388	0.17	0.7	0.5	1.0
rs12591436	15	*MTHFS*	1388	0.35	0.6	0.5	1.0
rs1081231	15	*MTHFS*	1387	0.18	0.8	0.5	1.0
rs12482346	21	*SLC19A1*	1388	0.47	0.1	0.5	1.0
rs2289093	2	*DNMT3A*	1388	0.28	0.2	0.5	1.0
rs1051296	21	*SLC19A1*	1375	0.47	0.1	0.5	1.0
rs282776	15	*MTHFS*	1388	0.36	1.0	0.5	1.0
rs2275566	1	*MTR*	1388	0.42	0.1	0.5	1.0
rs10165919	2	*ATIC*	1387	0.35	0.6	0.5	1.0
rs1256095	14	*MTHFD1*	1377	0.48	0.8	0.5	1.0
rs898436	15	*MTHFS*	1384	0.46	0.1	0.6	1.0
rs3740392	10	*AS3MT*	1385	0.29	0.1	0.6	1.0
rs600671	15	*MTHFS*	1387	0.46	0.1	0.6	1.0
rs4646350	17	*PEMT*	1388	0.36	0.6	0.6	1.0
rs12997662	2	*ATIC*	1387	0.33	0.7	0.6	1.0
rs11869600	17	*PEMT*	1386	0.38	0.3	0.6	1.0
rs4804122	19	*DNMT1*	1388	0.40	0.4	0.6	1.0
rs8081810	17	*PEMT*	1387	0.21	0.9	0.6	1.0
rs6485991	11	*FOLH1*	1382	0.16	0.6	0.6	1.0
rs34048824	2	*DNMT3A*	1387	0.50	1.0	0.6	1.0

Supplementary Table S5-6 (cont.) Uncorrected and corrected p-values for the interaction between D4Z4 methylation levels and selected SNPs in the one-carbon metabolism pathway in the Spanish Bladder Cancer/EPICURO Study

dbSNP ID (N=375)	Chromosome	Gene	N	MAF	*P* HWE	*P* LRT	Corrected *P* LRT
rs12987326	2	*DNMT3A*	1388	0.37	0.7	0.6	1.0
rs2987981	14	*MTHFD1*	1388	0.25	0.9	0.6	1.0
rs567754	5	*BHMT*	1387	0.29	0.9	0.6	1.0
rs443394	15	*MTHFS*	1388	0.41	0.3	0.6	1.0
rs1667627	14	*MTHFD2*	1387	0.46	0.2	0.6	1.0
rs12121543	1	*MTHFR*	1388	0.22	0.9	0.6	1.0
rs4646340	17	*PEMT*	1388	0.36	0.5	0.6	1.0
rs12999687	2	*DNMT3A*	1386	0.45	0.8	0.6	1.0
rs7253062	19	*DNMT1*	1388	0.39	0.7	0.6	1.0
rs11656215	17	*PEMT*	1388	0.45	0.4	0.6	1.0
rs853858	20	*DNMT3B*	1386	0.37	0.2	0.6	1.0
rs2275565	1	*MTR*	1388	0.20	0.6	0.6	1.0
rs8011839	14	*MTHFD1*	1388	0.18	0.4	0.6	1.0
rs12627639	21	*SLC19A1*	1387	0.21	0.5	0.6	1.0
rs11681447	2	*DNMT3A*	1386	0.28	0.2	0.6	1.0
NA	1	*MTR_01_2_i*	1388	0.17	0.7	0.6	1.0
rs12905663	15	*MTHFS*	1381	0.27	0.2	0.6	1.0
rs11687225	2	*ATIC*	1387	0.40	0.9	0.6	1.0
rs11887120	2	*DNMT3A*	1388	0.42	0.5	0.6	1.0
rs944422	21	*SLC19A1*	1384	0.35	0.9	0.6	1.0
rs282778	15	*MTHFS*	1388	0.27	0.8	0.6	1.0
rs2124344	17	*PEMT*	1387	0.36	0.7	0.6	1.0
rs4779141	15	*MTHFS*	1386	0.34	0.8	0.6	1.0
rs8101626	19	*DNMT1*	1388	0.39	0.7	0.6	1.0
rs9977111	21	*SLC19A1*	1355	0.33	0.07	0.6	1.0
rs7604425	2	*ATIC*	1388	0.35	0.7	0.6	1.0
rs10748835	10	*AS3MT*	1388	0.44	0.3	0.6	1.0
rs4532960	10	*AS3MT*	1387	0.44	0.3	0.6	1.0
rs282814	15	*MTHFS*	1388	0.21	0.8	0.6	1.0
rs202712	11	*FOLH1*	1387	0.23	0.7	0.6	1.0
rs7575625	2	*DNMT3A*	1388	0.47	0.9	0.6	1.0
rs8128676	21	*SLC19A1*	1350	0.22	1.0	0.6	1.0
rs1808119	2	*ATIC*	1388	0.20	1.0	0.6	1.0
rs6518253	21	*SLC19A1*	1387	0.46	0.3	0.7	1.0
rs749130	2	*DNMT3A*	1388	0.44	0.6	0.7	1.0
rs17285431	15	*MTHFS*	1388	0.17	0.1	0.7	1.0
rs5749131	22	*TCN2*	1388	0.43	0.2	0.7	1.0
rs16853834	2	*ATIC*	1388	0.16	1.0	0.7	1.0
rs4244599	17	*PEMT*	1371	0.46	0.4	0.7	1.0
rs6713377	2	*DNMT3A*	1387	0.47	0.9	0.7	1.0

Supplementary Table S5-6 (cont.) Uncorrected and corrected p-values for the interaction between D4Z4 methylation levels and selected SNPs in the one-carbon metabolism pathway in the Spanish Bladder Cancer/EPICURO Study

dbSNP ID (N=375)	Chromosome	Gene	N	MAF	*P* HWE	*P* LRT	Corrected *P* LRT
rs685487	15	*MTHFS*	1388	0.37	0.09	0.7	1.0
rs2987969	14	*MTHFD1*	1387	0.45	0.7	0.7	1.0
rs12899781	15	*MTHFS*	1383	0.17	0.06	0.7	1.0
rs734693	2	*DNMT3A*	1387	0.29	0.2	0.7	1.0
rs2289195	2	*DNMT3A*	1387	0.44	0.2	0.7	1.0
rs2983733	14	*MTHFD1*	1388	0.45	0.9	0.7	1.0
rs1076991	14	*MTHFD1*	1388	0.45	1.0	0.7	1.0
rs2983736	14	*MTHFD1*	1385	0.45	0.9	0.7	1.0
rs1473406	15	*MTHFS*	1386	0.15	0.9	0.7	1.0
rs1369703	2	*DNMT3A*	1388	0.45	0.2	0.7	1.0
rs10498034	2	*ATIC*	1388	0.17	1.0	0.7	1.0
rs8129350	21	*SLC19A1*	1387	0.35	0.9	0.7	1.0
rs8018032	14	*MTHFD1*	1388	0.45	0.7	0.7	1.0
rs1888533	21	*SLC19A1*	1388	0.48	0.3	0.7	1.0
rs748196	17	*PEMT*	1385	0.42	0.9	0.7	1.0
rs10839239	11	*FOLH1*	1386	0.23	0.6	0.7	1.0
rs11158538	14	*MTHFD1*	1385	0.45	0.9	0.7	1.0
rs1404774	2	*ATIC*	1380	0.21	0.4	0.7	1.0
rs11040416	11	*FOLH1*	1388	0.42	0.4	0.7	1.0
rs2288349	19	*DNMT1*	1387	0.39	0.5	0.7	1.0
rs4531931	2	*ATIC*	1387	0.31	0.5	0.7	1.0
rs6760069	2	*ATIC*	1387	0.14	0.5	0.7	1.0
rs1109859	17	*PEMT*	1367	0.18	0.8	0.7	1.0
rs617219	5	*BHMT*	1387	0.32	0.7	0.7	1.0
rs445263	15	*MTHFS*	1388	0.30	0.9	0.7	1.0
rs1809986	11	*FOLH1*	1388	0.37	1.0	0.7	1.0
rs6445606	3	*CHDH*	1388	0.29	0.2	0.8	1.0
rs376863	15	*MTHFS*	1366	0.50	0.9	0.8	1.0
rs660439	11	*FOLH1*	1384	0.23	0.7	0.8	1.0
rs750546	17	*PEMT*	1373	0.43	0.5	0.8	1.0
rs8003379	14	*MTHFD1*	1387	0.24	0.4	0.8	1.0
rs1801394	5	*MTRTR*	1398	0.49	0.8	0.8	1.0
rs7120743	11	*FOLH1*	1371	0.37	0.8	0.8	1.0
rs5753231	22	*TCN2*	1387	0.16	0.8	0.8	1.0
rs9836592	3	*CHDH*	1388	0.31	0.3	0.8	1.0
rs1464864	2	*ATIC*	1388	0.30	0.8	0.8	1.0
rs699517	18	*TYMS*	1387	0.31	0.6	0.8	1.0
rs1256112	14	*MTHFD1*	1388	0.40	0.4	0.8	1.0
rs11040106	11	*FOLH1*	1379	0.36	0.6	0.8	1.0
rs1059394	18	*TYMS*	1387	0.31	0.6	0.8	1.0

Supplementary Table S5-6 (cont.) Uncorrected and corrected p-values for the interaction between D4Z4 methylation levels and selected SNPs in the one-carbon metabolism pathway in the Spanish Bladder Cancer/EPICURO Study

dbSNP ID (N=375)	Chromosome	Gene	N	MAF	*P* HWE	*P* LRT	Corrected *P* LRT
rs1077965	15	*MTHFS*	1387	0.41	0.4	0.8	1.0
rs731991	22	*TCN2*	1343	0.48	0.1	0.8	1.0
rs7594432	2	*DNMT3A*	1388	0.45	0.3	0.8	1.0
rs378057	15	*MTHFS*	1387	0.14	1.0	0.8	1.0
rs12453139	17	*PEMT*	1386	0.26	0.9	0.8	1.0
rs6511677	19	*DNMT1*	1387	0.39	0.6	0.8	1.0
rs2790	18	*TYMS*	1384	0.20	0.7	0.8	1.0
rs1650697	5	*DHFR*	1385	0.23	0.1	0.8	1.0
rs1055345	21	*SLC19A1*	1387	0.29	0.3	0.8	1.0
rs2162560	19	*DNMT1*	1387	0.39	0.6	0.8	1.0
rs13427202	2	*DNMT3A*	1387	0.47	0.6	0.9	1.0
rs11694842	2	*DNMT3A*	1387	0.28	0.4	0.9	1.0
rs8128050	21	*SLC19A1*	1385	0.35	0.9	0.9	1.0
rs17824591	14	*MTHFD1*	1386	0.23	0.2	0.9	1.0
rs4393531	15	*MTHFS*	1386	0.48	0.4	0.9	1.0
rs7085104	10	*AS3MT*	1388	0.39	1.0	0.9	1.0
rs12898670	15	*MTHFS*	1387	0.32	0.9	0.9	1.0
rs234706	21	*CBS*	1388	0.32	0.1	0.9	1.0
rs4610054	2	*ATIC*	1383	0.38	1.0	0.9	1.0
rs10839295	11	*FOLH1*	1387	0.39	0.5	0.9	1.0
rs1917321	11	*FOLH1*	1380	0.50	0.2	0.9	1.0
rs7586294	2	*DNMT3A*	1387	0.47	0.5	0.9	1.0
rs4819138	21	*SLC19A1*	1388	0.39	0.8	0.9	1.0
rs166868	15	*MTHFS*	1387	0.36	1.0	0.9	1.0
rs11701960	21	*SLC19A1*	1387	0.17	1.0	0.9	1.0
rs8129445	21	*SLC19A1*	1388	0.32	0.9	0.9	1.0
rs6801605	3	*CHDH*	1388	0.38	0.6	0.9	1.0
rs11855092	15	*MTHFS*	1388	0.23	0.07	0.9	1.0
rs17284990	15	*MTHFS*	1388	0.22	0.5	0.9	1.0
rs9789571	2	*ATIC*	1388	0.42	1.0	0.9	1.0
rs282772	15	*MTHFS*	1388	0.13	0.3	0.9	1.0
rs1814175	11	*FOLH1*	1384	0.39	0.4	0.9	1.0
rs202700	11	*FOLH1*	1337	0.23	0.8	0.9	1.0
rs4673981	2	*ATIC*	1388	0.39	0.8	0.9	1.0
rs1023159	21	*SLC19A1*	1386	0.42	0.4	0.9	1.0
rs3862350	11	*FOLH1*	1360	0.39	0.2	0.9	1.0
rs2301955	22	*TCN2*	1386	0.43	0.3	0.9	1.0
rs6586282	21	*CBS*	1387	0.17	0.2	0.9	1.0
rs8128681	21	*SLC19A1*	1388	0.32	0.9	1.0	1.0
rs5749135	22	*TCN2*	1388	0.43	0.3	1.0	1.0

Supplementary Table S5-6 (cont.) Uncorrected and corrected p-values for the interaction between D4Z4 methylation levels and selected SNPs in the one-carbon metabolism pathway in the Spanish Bladder Cancer/EPICURO Study

dbSNP ID (N=375)	Chromosome	Gene	N	MAF	*P* HWE	*P* LRT	Corrected *P* LRT
rs9462856	6	*GNMT*	1388	0.41	0.9	1.0	1.0
rs767138	21	*SLC19A1*	1385	0.40	0.6	1.0	1.0
rs2838970	21	*SLC19A1*	1387	0.39	0.9	1.0	1.0
rs13036246	2	*DNMT3A*	1387	0.49	0.4	1.0	1.0
rs7279305	21	*SLC19A1*	1388	0.35	1.0	1.0	1.0
rs4476347	2	*ATIC*	1387	0.25	0.4	1.0	1.0
rs9621047	22	*TCN2*	1388	0.44	0.2	1.0	1.0
rs13002567	2	*DNMT3A*	1388	0.28	0.5	1.0	1.0
rs4778721	15	*MTHFS*	1388	0.23	0.2	1.0	1.0
rs2877078	21	*SLC19A1*	1376	0.39	0.7	1.0	1.0
rs2241807	3	*CHDH*	1388	0.43	0.7	1.0	1.0
rs4820888	22	*TCN2*	1387	0.47	0.1	1.0	1.0
rs582172	15	*MTHFS*	1388	0.41	0.6	1.0	1.0
rs2838977	21	*SLC19A1*	1386	0.39	0.9	1.0	1.0
rs4778719	15	*MTHFS*	1388	0.23	0.2	1.0	1.0
rs17279885	15	*MTHFS*	1388	0.21	0.7	1.0	1.0
rs17279753	15	*MTHFS*	1388	0.20	0.5	1.0	1.0

Supplementary Table S5-6 (cont.) Uncorrected and corrected p-values for the interaction between D4Z4 methylation levels and selected SNPs in the one-carbon metabolism pathway in the Spanish Bladder Cancer/EPICURO Study

SNPs modeled in dominant mode of inheritance							
dbSNP ID (N=495)	Chromosome	Gene	N	MAF	*P* HWE	*P* LRT	Corrected *P* LRT
rs12098986	11	*FOLH1*	1386	0.09	0.2	0.0007	0.2
rs12293923	11	*FOLH1*	1383	0.09	0.2	0.0007	0.2
rs7358352	11	*FOLH1*	1387	0.09	0.4	0.0008	0.2
rs650826	11	*FOLH1*	1386	0.09	0.2	0.0009	0.2
rs11040261	11	*FOLH1*	1388	0.09	0.4	0.001	0.2
rs598841	11	*FOLH1*	1383	0.09	0.2	0.001	0.2
rs632220	11	*FOLH1*	1388	0.09	0.4	0.001	0.2
rs1819409	11	*FOLH1*	1376	0.09	0.3	0.001	0.3
rs683680	11	*FOLH1*	1387	0.09	0.2	0.001	0.3
rs7926211	11	*FOLH1*	1385	0.09	0.2	0.001	0.3
rs7117025	11	*FOLH1*	1388	0.09	0.4	0.002	0.3
rs11040263	11	*FOLH1*	1385	0.09	0.2	0.002	0.4
rs4495895	11	*FOLH1*	1353	0.09	0.4	0.004	0.6
rs6087990	20	*DNMT3B*	1388	0.33	0.6	0.004	0.7
rs6495449	15	*MTHFS*	1388	0.11	0.09	0.005	0.7
rs2865908	11	*FOLH1*	1388	0.19	0.9	0.007	0.8
rs4094478	11	*FOLH1*	1365	0.20	0.7	0.007	0.8
rs10839210	11	*FOLH1*	1386	0.21	0.5	0.009	0.9
rs2305230	3	*ALDH1L1*	1387	0.19	0.7	0.01	0.9
rs10769558	11	*FOLH1*	1388	0.21	0.5	0.01	0.9
rs1847638	11	*FOLH1*	1335	0.21	1.0	0.01	0.9
rs11158542	14	*MTHFD1*	1388	0.29	0.8	0.01	0.9
rs3821353	2	*ATIC*	1388	0.21	0.4	0.01	0.9
rs6058869	20	*DNMT3B*	1388	0.33	0.7	0.01	1.0
rs6573559	14	*MTHFD1*	1388	0.29	1.0	0.02	1.0
rs11191457	10	*AS3MT*	1385	0.23	0.4	0.02	1.0
rs11040198	11	*FOLH1*	1381	0.21	0.2	0.02	1.0
rs2696923	11	*FOLH1*	1388	0.21	0.3	0.02	1.0
rs3772078	2	*ATIC*	1388	0.21	0.6	0.02	1.0
rs16853782	2	*ATIC*	1388	0.21	0.7	0.02	1.0
rs6058893	20	*DNMT3B*	1388	0.33	0.2	0.02	1.0
rs7929543	11	*FOLH1*	1388	0.08	1.0	0.02	1.0
rs4441015	11	*FOLH1*	1353	0.15	0.5	0.02	1.0
rs2696935	11	*FOLH1*	1388	0.21	0.4	0.02	1.0
rs7085854	10	*AS3MT*	1387	0.23	0.6	0.02	1.0
rs11627387	14	*MTHFD1*	1388	0.29	0.9	0.02	1.0
rs4911107	20	*DNMT3B*	1388	0.32	0.6	0.03	1.0
rs11607791	11	*FOLH1*	1387	0.07	1.0	0.03	1.0

Supplementary Table S5-6 (cont.) Uncorrected and corrected p-values for the interaction between D4Z4 methylation levels and selected SNPs in the one-carbon metabolism pathway in the Spanish Bladder Cancer/EPICURO Study

dbSNP ID (N=495)	Chromosome	Gene	N	MAF	*P* HWE	*P* LRT	Corrected *P* LRT
rs12438477	15	*MTHFS*	1387	0.36	0.4	0.03	1.0
rs6058897	20	*DNMT3B*	1388	0.43	0.2	0.03	1.0
rs12913164	15	*MTHFS*	1388	0.07	0.8	0.03	1.0
rs2281603	14	*MTHFD1*	1388	0.19	0.2	0.04	1.0
rs11040421	11	*FOLH1*	1388	0.15	0.6	0.04	1.0
rs9974061	21	*SLC19A1*	1388	0.18	1.0	0.04	1.0
rs2424928	20	*DNMT3B*	1388	0.39	0.2	0.04	1.0
rs2838958	21	*SLC19A1*	1385	0.45	0.7	0.04	1.0
rs2035027	15	*MTHFS*	1388	0.16	0.5	0.04	1.0
rs9323450	14	*MTHFD1*	1388	0.30	0.8	0.05	1.0
rs11634787	15	*MTHFS*	1388	0.09	0.3	0.05	1.0
rs16853826	2	*ATIC*	1386	0.13	0.9	0.05	1.0
rs2424921	20	*DNMT3B*	1388	0.39	0.3	0.05	1.0
rs2424922	20	*DNMT3B*	1387	0.39	0.2	0.06	1.0
rs7111711	11	*FOLH1*	1388	0.25	0.9	0.06	1.0
rs12900076	15	*MTHFS*	1385	0.08	0.6	0.06	1.0
rs16971450	15	*MTHFS*	1387	0.16	0.4	0.06	1.0
rs865646	5	*DHFR*	1340	0.34	0.4	0.06	1.0
rs2236224	14	*MTHFD1*	1388	0.36	0.1	0.06	1.0
rs6058891	20	*DNMT3B*	1386	0.39	0.2	0.06	1.0
rs4779165	15	*MTHFS*	1387	0.16	0.4	0.06	1.0
rs10839229	11	*FOLH1*	1386	0.08	0.3	0.06	1.0
rs4817577	21	*GART*	1388	0.34	0.9	0.06	1.0
rs10839224	11	*FOLH1*	1388	0.08	0.3	0.06	1.0
rs7107178	11	*FOLH1*	1387	0.25	1.0	0.07	1.0
rs7605753	2	*DNMT3A*	1388	0.47	0.6	0.07	1.0
rs6749992	2	*DNMT3A*	1388	0.47	0.8	0.07	1.0
rs10839296	11	*FOLH1*	1371	0.25	0.8	0.07	1.0
rs559062	1	*CTH*	1388	0.22	0.6	0.07	1.0
rs12898642	15	*MTHFS*	1387	0.43	0.2	0.07	1.0
rs585800	5	*BHMT*	1388	0.26	0.05	0.07	1.0
rs6141813	20	*DNMT3B*	1388	0.13	0.8	0.07	1.0
rs7283354	21	*GART*	1388	0.34	0.9	0.07	1.0
rs4817579	21	*GART*	1388	0.35	1.0	0.08	1.0
rs16906158	11	*FOLH1*	1387	0.08	0.6	0.08	1.0
rs6087988	20	*DNMT3B*	1388	0.20	0.6	0.08	1.0
rs910085	20	*DNMT3B*	1387	0.29	0.5	0.09	1.0
rs6058883	20	*DNMT3B*	1387	0.39	0.3	0.09	1.0
rs2424914	20	*DNMT3B*	1388	0.39	0.4	0.09	1.0
rs4818789	21	*SLC19A1*	1388	0.25	0.5	0.1	1.0

Supplementary Table S5-6 (cont.) Uncorrected and corrected p-values for the interaction between D4Z4 methylation levels and selected SNPs in the one-carbon metabolism pathway in the Spanish Bladder Cancer/EPICURO Study

dbSNP ID (N=495)	Chromosome	Gene	N	MAF	*P* HWE	*P* LRT	Corrected *P* LRT
rs2834233	21	*GART*	1387	0.08	0.5	0.1	1.0
rs13317328	3	*CHDH*	1388	0.10	0.1	0.1	1.0
rs1164681	11	*FOLH1*	1388	0.12	0.5	0.1	1.0
rs4646410	17	*PEMT*	1384	0.31	0.9	0.1	1.0
rs12592743	15	*MTHFS*	1388	0.09	0.5	0.1	1.0
rs34033751	11	*FOLH1*	1371	0.11	0.4	0.1	1.0
rs2424913	20	*DNMT3B*	1387	0.37	0.2	0.1	1.0
rs2424906	20	*DNMT3B*	1388	0.38	0.2	0.1	1.0
rs9890064	17	*PEMT*	1388	0.43	0.4	0.1	1.0
rs202676	11	*FOLH1*	1380	0.17	0.8	0.1	1.0
rs2838956	21	*SLC19A1*	1373	0.44	0.07	0.1	1.0
rs372447	15	*MTHFS*	1388	0.38	0.9	0.1	1.0
rs9606756	22	*TCN2*	1388	0.10	0.7	0.1	1.0
rs11852515	15	*MTHFS*	1388	0.12	0.7	0.1	1.0
rs679470	11	*FOLH1*	1387	0.17	0.6	0.1	1.0
rs7583409	2	*DNMT3A*	1387	0.35	0.4	0.1	1.0
rs3897953	15	*MTHFS*	1388	0.09	0.8	0.1	1.0
rs4819128	21	*SLC19A1*	1388	0.44	0.2	0.1	1.0
rs7587636	2	*DNMT3A*	1388	0.45	0.1	0.1	1.0
rs1051266	21	*SLC19A1*	1388	0.45	0.2	0.1	1.0
rs3788200	21	*SLC19A1*	1388	0.45	0.3	0.1	1.0
rs9910747	17	*PEMT*	1388	0.07	0.4	0.1	1.0
rs282792	15	*MTHFS*	1388	0.39	0.6	0.1	1.0
rs10163099	15	*MTHFS*	1385	0.26	0.1	0.1	1.0
rs6495446	15	*MTHFS*	1387	0.26	0.1	0.1	1.0
rs13401241	2	*DNMT3A*	1388	0.45	0.1	0.1	1.0
rs11040432	11	*FOLH1*	1387	0.08	1.0	0.1	1.0
rs648372	11	*FOLH1*	1370	0.17	0.5	0.2	1.0
rs1404772	2	*ATIC*	1388	0.08	0.4	0.2	1.0
rs914232	21	*SLC19A1*	1387	0.44	0.2	0.2	1.0
rs1846285	11	*FOLH1*	1385	0.17	0.2	0.2	1.0
rs16988828	22	*TCN2*	1388	0.09	0.2	0.2	1.0
rs12903985	15	*MTHFS*	1388	0.28	0.4	0.2	1.0
rs4911108	20	*DNMT3B*	1384	0.29	0.7	0.2	1.0
rs1380642	15	*MTHFS*	1388	0.18	0.3	0.2	1.0
rs914231	21	*SLC19A1*	1383	0.44	0.2	0.2	1.0
rs8659	5	*MTRR*	1386	0.35	0.3	0.2	1.0
rs10418	22	*TCN2*	1367	0.21	0.7	0.2	1.0
rs663649	1	*CTH*	1388	0.31	0.9	0.2	1.0
rs7124266	11	*FOLH1*	1388	0.30	0.6	0.2	1.0

Supplementary Table S5-6 (cont.) Uncorrected and corrected p-values for the interaction between D4Z4 methylation levels and selected SNPs in the one-carbon metabolism pathway in the Spanish Bladder Cancer/EPICURO Study

dbSNP ID (N=495)	Chromosome	Gene	N	MAF	*P* HWE	*P* LRT	Corrected *P* LRT
rs473334	1	*CTH*	1388	0.31	0.9	0.2	1.0
rs6706415	2	*ATIC*	1388	0.30	0.4	0.2	1.0
rs10948059	6	*GNMT*	1370	0.48	0.8	0.2	1.0
rs4911263	20	*DNMT3B*	1388	0.32	1.0	0.2	1.0
rs1046778	10	*AS3MT*	1388	0.33	0.8	0.2	1.0
rs7102641	11	*FOLH1*	1385	0.08	0.4	0.2	1.0
rs10197653	2	*ATIC*	1388	0.29	0.1	0.2	1.0
rs4819130	21	*SLC19A1*	1384	0.45	0.2	0.2	1.0
rs2838964	21	*SLC19A1*	1388	0.07	1.0	0.2	1.0
rs7215833	17	*PEMT*	1388	0.35	0.5	0.2	1.0
rs7951180	11	*FOLH1*	1369	0.17	0.4	0.2	1.0
rs4646341	17	*PEMT*	1385	0.36	0.5	0.2	1.0
rs8074074	17	*PEMT*	1386	0.29	0.8	0.2	1.0
rs1802059	5	*MTRR*	1387	0.37	0.4	0.2	1.0
rs3177999	21	*SLC19A1*	1380	0.45	0.2	0.2	1.0
rs12614943	2	*ATIC*	1388	0.28	1.0	0.2	1.0
rs10498036	2	*ATIC*	1388	0.40	0.4	0.2	1.0
rs7934591	11	*FOLH1*	1388	0.08	0.8	0.2	1.0
rs9897362	17	*PEMT*	1388	0.06	0.3	0.2	1.0
rs10400277	11	*FOLH1*	1368	0.13	0.6	0.2	1.0
rs2330183	21	*SLC19A1*	1358	0.44	0.05	0.2	1.0
rs10197559	2	*ATIC*	1380	0.29	0.9	0.2	1.0
rs914238	21	*SLC19A1*	1388	0.49	0.4	0.2	1.0
rs7220132	17	*PEMT*	1388	0.29	0.7	0.2	1.0
rs9282690	3	*ALDH1L1*	1388	0.07	0.8	0.2	1.0
rs3783	17	*SHMT1*	1398	0.26	0.6	0.2	1.0
rs11040390	11	*FOLH1*	1388	0.08	1.0	0.2	1.0
NA	1	*MTR_01_OR*	1389	0.16	0.7	0.2	1.0
rs2838961	21	*SLC19A1*	1388	0.34	1.0	0.2	1.0
rs9835128	3	*CHDH*	1387	0.17	0.4	0.2	1.0
rs12222221	11	*FOLH1*	1388	0.08	1.0	0.2	1.0
rs4646385	17	*PEMT*	1388	0.43	0.5	0.2	1.0
rs17279286	15	*MTHFS*	1387	0.05	0.7	0.2	1.0
rs7117247	11	*FOLH1*	1388	0.07	0.1	0.2	1.0
rs17745484	2	*DNMT3A*	1387	0.35	0.8	0.2	1.0
rs7604984	2	*ATIC*	1388	0.40	0.4	0.2	1.0
rs7560488	2	*DNMT3A*	1333	0.47	0.2	0.2	1.0
rs4479310	17	*PEMT*	1388	0.29	0.6	0.3	1.0
rs1256107	14	*MTHFD1*	1387	0.48	0.7	0.3	1.0
rs1051298	21	*SLC19A1*	1387	0.46	0.3	0.3	1.0

Supplementary Table S5-6 (cont.) Uncorrected and corrected p-values for the interaction between D4Z4 methylation levels and selected SNPs in the one-carbon metabolism pathway in the Spanish Bladder Cancer/EPICURO Study

dbSNP ID (N=495)	Chromosome	Gene	N	MAF	*P* HWE	*P* LRT	Corrected *P* LRT
rs10179873	2	*ATIC*	1388	0.30	0.4	0.3	1.0
rs4646359	17	*PEMT*	1388	0.45	0.3	0.3	1.0
rs7177659	15	*MTHFS*	1387	0.50	0.08	0.3	1.0
rs1950902	14	*MTHFD1*	1388	0.14	0.4	0.3	1.0
rs12416687	10	*AS3MT*	1388	0.28	0.06	0.3	1.0
rs7113075	11	*FOLH1*	1387	0.08	0.8	0.3	1.0
rs1256095	14	*MTHFD1*	1377	0.48	0.8	0.3	1.0
rs740234	22	*TCN2*	1388	0.23	0.8	0.3	1.0
rs16999714	19	*DNMT1*	1386	0.21	0.1	0.3	1.0
rs3740392	10	*AS3MT*	1385	0.29	0.1	0.3	1.0
rs6711622	2	*DNMT3A*	1388	0.44	1.0	0.3	1.0
rs4673965	2	*ATIC*	1388	0.40	1.0	0.3	1.0
rs3785499	17	*PEMT*	1388	0.47	0.8	0.3	1.0
rs12591436	15	*MTHFS*	1388	0.35	0.6	0.3	1.0
rs10380	5	*MTRR*	1388	0.10	0.4	0.3	1.0
rs2834235	21	*GART*	1388	0.38	0.7	0.3	1.0
rs11892429	2	*ATIC*	1388	0.29	0.7	0.3	1.0
rs2066470	1	*MTHFR*	1384	0.09	0.2	0.3	1.0
rs11040353	11	*FOLH1*	1387	0.08	0.8	0.3	1.0
rs4804122	19	*DNMT1*	1388	0.40	0.4	0.3	1.0
rs435689	15	*MTHFS*	1388	0.49	0.9	0.3	1.0
rs7946	17	*PEMT*	1387	0.29	0.4	0.3	1.0
rs600671	15	*MTHFS*	1387	0.46	0.1	0.3	1.0
rs9332	5	*MTRR*	1388	0.12	0.2	0.3	1.0
rs12613	21	*CBS*	1388	0.08	1.0	0.3	1.0
rs7217764	17	*PEMT*	1388	0.05	1.0	0.3	1.0
rs898436	15	*MTHFS*	1384	0.46	0.1	0.3	1.0
rs1081231	15	*MTHFS*	1387	0.18	0.8	0.3	1.0
rs4646350	17	*PEMT*	1388	0.36	0.6	0.3	1.0
rs4779140	15	*MTHFS*	1387	0.48	0.6	0.3	1.0
rs17291414	19	*DNMT1*	1388	0.27	0.5	0.3	1.0
rs1667627	14	*MTHFD2*	1387	0.46	0.2	0.3	1.0
rs770144	15	*MTHFS*	1388	0.20	0.3	0.3	1.0
rs10501325	11	*FOLH1*	1388	0.07	0.1	0.3	1.0
rs35020344	14	*MTHFD1*	1387	0.47	0.7	0.3	1.0
rs4646340	17	*PEMT*	1388	0.36	0.5	0.3	1.0
rs515064	1	*CTH*	1388	0.36	0.8	0.3	1.0
rs2372535	2	*ATIC*	1388	0.15	1.0	0.3	1.0
rs282778	15	*MTHFS*	1388	0.27	0.8	0.3	1.0
rs1983462	2	*ATIC*	1388	0.30	0.3	0.3	1.0

Supplementary Table S5-6 (cont.) Uncorrected and corrected p-values for the interaction between D4Z4 methylation levels and selected SNPs in the one-carbon metabolism pathway in the Spanish Bladder Cancer/EPICURO Study

dbSNP ID (N=495)	Chromosome	Gene	N	MAF	*P* HWE	*P* LRT	Corrected *P* LRT
rs7177027	15	*MTHFS*	1388	0.24	0.08	0.3	1.0
rs3760188	17	*PEMT*	1388	0.46	0.6	0.3	1.0
rs10165919	2	*ATIC*	1387	0.35	0.6	0.3	1.0
rs2838965	21	*SLC19A1*	1384	0.42	0.05	0.3	1.0
rs4646344	17	*PEMT*	1388	0.47	0.7	0.3	1.0
rs12905663	15	*MTHFS*	1381	0.27	0.2	0.3	1.0
rs2154583	21	*GART*	1384	0.39	0.7	0.3	1.0
rs853858	20	*DNMT3B*	1386	0.37	0.2	0.3	1.0
rs6517178	21	*GART*	1388	0.39	0.7	0.3	1.0
rs8101626	19	*DNMT1*	1388	0.39	0.7	0.3	1.0
rs2124344	17	*PEMT*	1387	0.36	0.7	0.3	1.0
rs1956545	14	*MTHFD1*	1387	0.08	0.5	0.3	1.0
rs3755817	3	*CHDH*	1387	0.30	0.5	0.3	1.0
rs2275565	1	*MTR*	1388	0.20	0.6	0.3	1.0
rs944422	21	*SLC19A1*	1384	0.35	0.9	0.3	1.0
rs12999687	2	*DNMT3A*	1386	0.45	0.8	0.3	1.0
rs12482346	21	*SLC19A1*	1388	0.47	0.1	0.3	1.0
rs4820887	22	*TCN2*	1388	0.09	0.6	0.3	1.0
rs3862342	11	*FOLH1*	1386	0.28	0.8	0.4	1.0
rs8128676	21	*SLC19A1*	1350	0.22	1.0	0.4	1.0
rs1460177	15	*MTHFS*	1388	0.09	0.4	0.4	1.0
rs7759302	6	*GNMT*	1388	0.06	1.0	0.4	1.0
rs3788190	21	*SLC19A1*	1387	0.47	0.1	0.4	1.0
rs2424932	20	*DNMT3B*	1387	0.43	0.5	0.4	1.0
rs1979276	17	*SHMT1*	1395	0.30	1.0	0.4	1.0
NA	1	*MTR_01_2_i*	1388	0.17	0.7	0.4	1.0
rs7575625	2	*DNMT3A*	1388	0.47	0.9	0.4	1.0
rs1256142	14	*MTHFD1*	1388	0.44	0.07	0.4	1.0
rs1805087	1	*MTR*	1388	0.17	0.7	0.4	1.0
rs2236225	14	*MTHFD1*	1387	0.43	0.2	0.4	1.0
rs11687225	2	*ATIC*	1387	0.40	0.9	0.4	1.0
rs663465	1	*CTH*	1387	0.41	0.4	0.4	1.0
rs7604425	2	*ATIC*	1388	0.35	0.7	0.4	1.0
rs8034036	15	*MTHFS*	1386	0.11	0.09	0.4	1.0
rs2289195	2	*DNMT3A*	1387	0.44	0.2	0.4	1.0
rs202718	11	*FOLH1*	1388	0.15	0.9	0.4	1.0
rs16971253	15	*MTHFS*	1387	0.10	1.0	0.4	1.0
rs8015278	14	*MTHFD1*	1388	0.07	0.8	0.4	1.0
rs8129350	21	*SLC19A1*	1387	0.35	0.9	0.4	1.0
rs9621049	22	*TCN2*	1388	0.10	0.8	0.4	1.0

Supplementary Table S5-6 (cont.) Uncorrected and corrected p-values for the interaction between D4Z4 methylation levels and selected SNPs in the one-carbon metabolism pathway in the Spanish Bladder Cancer/EPICURO Study

dbSNP ID (N=495)	Chromosome	Gene	N	MAF	*P* HWE	*P* LRT	Corrected *P* LRT
rs1888533	21	*SLC19A1*	1388	0.48	0.3	0.4	1.0
rs10460566	2	*DNMT3A*	1388	0.27	0.3	0.4	1.0
rs11677670	2	*DNMT3A*	1382	0.17	0.5	0.4	1.0
rs1074390	15	*MTHFS*	1388	0.37	0.6	0.4	1.0
rs6495441	15	*MTHFS*	1388	0.24	0.3	0.4	1.0
rs202712	11	*FOLH1*	1387	0.23	0.7	0.4	1.0
rs6713377	2	*DNMT3A*	1387	0.47	0.9	0.4	1.0
rs3740394	10	*AS3MT*	1387	0.12	0.3	0.4	1.0
rs2289093	2	*DNMT3A*	1388	0.28	0.2	0.4	1.0
rs7253062	19	*DNMT1*	1388	0.39	0.7	0.4	1.0
rs10932605	2	*ATIC*	1388	0.14	0.3	0.4	1.0
rs4531931	2	*ATIC*	1387	0.31	0.5	0.4	1.0
rs16971249	15	*MTHFS*	1388	0.07	0.08	0.4	1.0
rs1109859	17	*PEMT*	1367	0.18	0.8	0.4	1.0
rs1979277	17	*SHMT1*	1398	0.27	0.8	0.4	1.0
rs10509760	10	*AS3MT*	1388	0.12	0.3	0.4	1.0
rs1888530	21	*SLC19A1*	1358	0.47	0.07	0.4	1.0
rs9001	3	*CHDH*	1385	0.10	0.3	0.4	1.0
rs4532960	10	*AS3MT*	1387	0.44	0.3	0.4	1.0
rs11656215	17	*PEMT*	1388	0.45	0.4	0.4	1.0
rs3893350	15	*MTHFS*	1388	0.13	0.1	0.4	1.0
rs734693	2	*DNMT3A*	1387	0.29	0.2	0.4	1.0
rs4924922	17	*PEMT*	1388	0.36	0.3	0.4	1.0
rs8923	15	*MTHFS*	1388	0.08	0.1	0.4	1.0
rs10748835	10	*AS3MT*	1388	0.44	0.3	0.4	1.0
rs4244599	17	*PEMT*	1371	0.46	0.4	0.4	1.0
rs12373907	21	*SLC19A1*	1387	0.38	0.4	0.5	1.0
rs617219	5	*BHMT*	1387	0.32	0.7	0.5	1.0
rs11683424	2	*DNMT3A*	1388	0.12	0.5	0.5	1.0
rs4817580	21	*GART*	1387	0.10	0.5	0.5	1.0
rs11681447	2	*DNMT3A*	1386	0.28	0.2	0.5	1.0
rs749130	2	*DNMT3A*	1388	0.44	0.6	0.5	1.0
rs8081810	17	*PEMT*	1387	0.21	0.9	0.5	1.0
rs8074191	17	*PEMT*	1372	0.28	0.9	0.5	1.0
rs2288350	19	*DNMT1*	1387	0.08	0.6	0.5	1.0
rs2288349	19	*DNMT1*	1387	0.39	0.5	0.5	1.0
rs10519256	15	*MTHFS*	1388	0.11	0.1	0.5	1.0
rs10839239	11	*FOLH1*	1386	0.23	0.6	0.5	1.0
rs12797843	11	*FOLH1*	1388	0.13	0.6	0.5	1.0
rs1880580	15	*MTHFS*	1388	0.30	0.09	0.5	1.0

Supplementary Table S5-6 (cont.) Uncorrected and corrected p-values for the interaction between D4Z4 methylation levels and selected SNPs in the one-carbon metabolism pathway in the Spanish Bladder Cancer/EPICURO Study

dbSNP ID (N=495)	Chromosome	Gene	N	MAF	*P* HWE	*P* LRT	Corrected *P* LRT
rs34048824	2	*DNMT3A*	1387	0.50	1.0	0.5	1.0
rs750546	17	*PEMT*	1373	0.43	0.5	0.5	1.0
rs12987326	2	*DNMT3A*	1388	0.37	0.7	0.5	1.0
rs11191439	10	*AS3MT*	1387	0.12	0.2	0.5	1.0
rs4902278	14	*MTHFD1*	1383	0.07	0.6	0.5	1.0
rs567754	5	*BHMT*	1387	0.29	0.9	0.5	1.0
rs1076991	14	*MTHFD1*	1388	0.45	1.0	0.5	1.0
NA	1	*MTHFR_02_OR*	1285	0.39	0.4	0.5	1.0
rs8068641	17	*PEMT*	1382	0.11	0.4	0.5	1.0
rs4369857	2	*ATIC*	1388	0.05	0.7	0.5	1.0
rs7563206	2	*ATIC*	1387	0.47	0.6	0.5	1.0
rs282814	15	*MTHFS*	1388	0.21	0.8	0.5	1.0
rs8111085	19	*DNMT1*	1387	0.08	0.6	0.5	1.0
rs1256114	14	*MTHFD1*	1387	0.11	0.7	0.5	1.0
rs11158538	14	*MTHFD1*	1385	0.45	0.9	0.5	1.0
rs3800292	6	*GNMT*	1388	0.06	0.8	0.5	1.0
rs8019804	14	*MTHFD1*	1388	0.07	1.0	0.5	1.0
rs3893384	15	*MTHFS*	1388	0.42	0.5	0.5	1.0
rs1051296	21	*SLC19A1*	1375	0.47	0.1	0.5	1.0
rs6750194	2	*ATIC*	1375	0.08	0.4	0.5	1.0
rs660439	11	*FOLH1*	1384	0.23	0.7	0.5	1.0
rs897453	17	*PEMT*	1384	0.49	0.6	0.5	1.0
rs17209637	15	*MTHFS*	1384	0.25	1.0	0.5	1.0
rs11672909	19	*DNMT1*	1387	0.08	0.6	0.5	1.0
rs11892646	2	*DNMT3A*	1387	0.12	0.6	0.5	1.0
rs12148881	15	*MTHFS*	1387	0.27	0.2	0.5	1.0
rs1465825	2	*DNMT3A*	1387	0.27	0.3	0.5	1.0
rs9977111	21	*SLC19A1*	1355	0.33	0.07	0.5	1.0
rs699517	18	*TYMS*	1387	0.31	0.6	0.5	1.0
rs7144437	14	*MTHFD1*	1387	0.07	0.6	0.5	1.0
rs1077965	15	*MTHFS*	1387	0.41	0.4	0.5	1.0
rs4779141	15	*MTHFS*	1386	0.34	0.8	0.6	1.0
rs6518253	21	*SLC19A1*	1387	0.46	0.3	0.6	1.0
rs1256112	14	*MTHFD1*	1388	0.40	0.4	0.6	1.0
rs378057	15	*MTHFS*	1387	0.14	1.0	0.6	1.0
rs4925048	17	*PEMT*	1386	0.09	0.1	0.6	1.0
rs12910340	15	*MTHFS*	1388	0.42	1.0	0.6	1.0
rs10418707	19	*DNMT1*	1387	0.08	0.6	0.6	1.0
rs2586153	15	*MTHFS*	1374	0.15	0.4	0.6	1.0
rs17556442	11	*FOLH1*	1384	0.06	0.05	0.6	1.0

Supplementary Table S5-6 (cont.) Uncorrected and corrected p-values for the interaction between D4Z4 methylation levels and selected SNPs in the one-carbon metabolism pathway in the Spanish Bladder Cancer/EPICURO Study

dbSNP ID (N=495)	Chromosome	Gene	N	MAF	*P* HWE	*P* LRT	Corrected *P* LRT
rs1059394	18	*TYMS*	1387	0.31	0.6	0.6	1.0
rs17211644	15	*MTHFS*	1388	0.10	0.7	0.6	1.0
rs8128050	21	*SLC19A1*	1385	0.35	0.9	0.6	1.0
rs4804494	19	*DNMT1*	1387	0.08	0.6	0.6	1.0
rs2115536	15	*MTHFS*	1388	0.50	0.05	0.6	1.0
rs10498034	2	*ATIC*	1388	0.17	1.0	0.6	1.0
rs4804490	19	*DNMT1*	1386	0.08	0.6	0.6	1.0
rs1055345	21	*SLC19A1*	1387	0.29	0.3	0.6	1.0
rs8971	21	*GART*	1386	0.26	0.8	0.6	1.0
rs5749131	22	*TCN2*	1388	0.43	0.2	0.6	1.0
rs12899781	15	*MTHFS*	1383	0.17	0.06	0.6	1.0
rs2790	18	*TYMS*	1384	0.20	0.7	0.6	1.0
rs1020697	17	*PEMT*	1361	0.10	0.3	0.6	1.0
rs2983733	14	*MTHFD1*	1388	0.45	0.9	0.6	1.0
rs1650697	5	*DHFR*	1385	0.23	0.1	0.6	1.0
rs2115540	15	*MTHFS*	1387	0.50	0.05	0.6	1.0
rs8041943	15	*MTHFS*	1385	0.42	0.6	0.6	1.0
rs1464864	2	*ATIC*	1388	0.30	0.8	0.6	1.0
rs282795	15	*MTHFS*	1387	0.32	0.7	0.6	1.0
rs2987981	14	*MTHFD1*	1388	0.25	0.9	0.6	1.0
rs7594432	2	*DNMT3A*	1388	0.45	0.3	0.6	1.0
rs17824591	14	*MTHFD1*	1386	0.23	0.2	0.6	1.0
rs282776	15	*MTHFS*	1388	0.36	1.0	0.6	1.0
rs6760069	2	*ATIC*	1387	0.14	0.5	0.6	1.0
rs2983736	14	*MTHFD1*	1385	0.45	0.9	0.6	1.0
rs17285431	15	*MTHFS*	1388	0.17	0.1	0.6	1.0
rs437302	20	*DNMT3B*	1388	0.09	1.0	0.6	1.0
rs2834231	21	*GART*	1388	0.26	0.7	0.6	1.0
rs11158540	14	*MTHFD1*	1388	0.34	0.9	0.6	1.0
rs2116940	19	*DNMT1*	1387	0.08	0.6	0.6	1.0
rs4610054	2	*ATIC*	1383	0.38	1.0	0.6	1.0
rs2834232	21	*GART*	1387	0.26	0.7	0.6	1.0
rs4804125	19	*DNMT1*	1387	0.08	0.6	0.6	1.0
rs7174668	15	*MTHFS*	1388	0.20	0.2	0.6	1.0
rs11658944	17	*PEMT*	1387	0.05	0.7	0.6	1.0
rs2733106	15	*MTHFS*	1383	0.15	0.5	0.6	1.0
rs2586154	15	*MTHFS*	1388	0.15	0.5	0.6	1.0
rs166868	15	*MTHFS*	1387	0.36	1.0	0.7	1.0
rs6511677	19	*DNMT1*	1387	0.39	0.6	0.7	1.0
rs7581217	2	*DNMT3A*	1388	0.39	0.2	0.7	1.0

Supplementary Table S5-6 (cont.) Uncorrected and corrected p-values for the interaction between D4Z4 methylation levels and selected SNPs in the one-carbon metabolism pathway in the Spanish Bladder Cancer/EPICURO Study

dbSNP ID (N=495)	Chromosome	Gene	N	MAF	*P* HWE	*P* LRT	Corrected *P* LRT
rs11869600	17	*PEMT*	1386	0.38	0.3	0.7	1.0
rs9305012	19	*DNMT1*	1387	0.08	0.6	0.7	1.0
rs12121543	1	*MTHFR*	1388	0.22	0.9	0.7	1.0
rs1917321	11	*FOLH1*	1380	0.50	0.2	0.7	1.0
rs2424908	20	*DNMT3B*	1388	0.17	0.7	0.7	1.0
rs11855092	15	*MTHFS*	1388	0.23	0.1	0.7	1.0
rs4393531	15	*MTHFS*	1386	0.48	0.4	0.7	1.0
rs12627639	21	*SLC19A1*	1387	0.21	0.5	0.7	1.0
rs2409495	21	*GART*	1388	0.19	0.05	0.7	1.0
rs12462004	19	*DNMT1*	1386	0.08	0.6	0.7	1.0
rs11040416	11	*FOLH1*	1388	0.42	0.4	0.7	1.0
rs2289209	3	*CHDH*	1388	0.05	1.0	0.7	1.0
rs685487	15	*MTHFS*	1388	0.37	0.1	0.7	1.0
rs17728676	11	*FOLH1*	1387	0.07	0.1	0.7	1.0
rs2162560	19	*DNMT1*	1387	0.39	0.6	0.7	1.0
rs8112895	19	*DNMT1*	1388	0.08	0.6	0.7	1.0
rs6801605	3	*CHDH*	1388	0.38	0.6	0.7	1.0
rs2586182	15	*MTHFS*	1388	0.15	0.4	0.7	1.0
rs4646404	17	*PEMT*	1383	0.36	0.3	0.7	1.0
rs11694842	2	*DNMT3A*	1387	0.28	0.4	0.7	1.0
rs17284990	15	*MTHFS*	1388	0.22	0.5	0.7	1.0
rs204942	15	*MTHFS*	1388	0.20	0.1	0.7	1.0
rs1880586	2	*ATIC*	1387	0.47	0.6	0.7	1.0
rs234706	21	*CBS*	1388	0.32	0.1	0.7	1.0
rs1917311	11	*FOLH1*	1353	0.40	0.4	0.7	1.0
rs1369703	2	*DNMT3A*	1388	0.45	0.2	0.7	1.0
rs1081235	15	*MTHFS*	1388	0.19	0.3	0.7	1.0
rs2267163	22	*TCN2*	1386	0.43	0.2	0.7	1.0
rs1404774	2	*ATIC*	1380	0.21	0.4	0.7	1.0
rs8128681	21	*SLC19A1*	1388	0.32	0.9	0.7	1.0
rs8011839	14	*MTHFD1*	1388	0.18	0.4	0.7	1.0
rs1801133	1	*MTHFR*	1388	0.40	0.5	0.7	1.0
NA	1	*MTHFR_02_2_i*	1388	0.40	0.5	0.7	1.0
rs1801198	22	*TCN2*	1387	0.43	0.3	0.7	1.0
rs282772	15	*MTHFS*	1388	0.13	0.3	0.8	1.0
rs9789571	2	*ATIC*	1388	0.42	1.0	0.8	1.0
rs2275566	1	*MTR*	1388	0.42	0.1	0.8	1.0
rs588458	11	*FOLH1*	1367	0.38	0.3	0.8	1.0
rs1814175	11	*FOLH1*	1384	0.39	0.4	0.8	1.0
rs16853834	2	*ATIC*	1388	0.16	1.0	0.8	1.0

Supplementary Table S5-6 (cont.) Uncorrected and corrected p-values for the interaction between D4Z4 methylation levels and selected SNPs in the one-carbon metabolism pathway in the Spanish Bladder Cancer/EPICURO Study

dbSNP ID (N=495)	Chromosome	Gene	N	MAF	*P* HWE	*P* LRT	Corrected *P* LRT
rs6485991	11	*FOLH1*	1382	0.16	0.6	0.8	1.0
rs7111215	11	*FOLH1*	1368	0.40	0.2	0.8	1.0
rs3774616	3	*CHDH*	1388	0.05	1.0	0.8	1.0
rs3862350	11	*FOLH1*	1360	0.39	0.2	0.8	1.0
rs2733103	15	*MTHFS*	1387	0.15	0.2	0.8	1.0
rs12898670	15	*MTHFS*	1387	0.32	0.9	0.8	1.0
rs4434082	21	*SLC19A1*	1387	0.21	0.9	0.8	1.0
rs11085720	19	*DNMT1*	1388	0.42	0.5	0.8	1.0
rs202700	11	*FOLH1*	1337	0.23	0.8	0.8	1.0
rs445263	15	*MTHFS*	1388	0.30	0.9	0.8	1.0
rs2838969	21	*SLC19A1*	1388	0.07	1.0	0.8	1.0
rs748196	17	*PEMT*	1385	0.42	0.9	0.8	1.0
rs12453139	17	*PEMT*	1386	0.26	0.9	0.8	1.0
rs3818239	14	*MTHFD1*	1380	0.13	0.9	0.8	1.0
rs9976878	21	*SLC19A1*	1387	0.21	0.8	0.8	1.0
rs6586282	21	*CBS*	1387	0.17	0.2	0.8	1.0
rs4819138	21	*SLC19A1*	1388	0.39	0.8	0.8	1.0
rs2838973	21	*SLC19A1*	1388	0.21	1.0	0.8	1.0
rs10839295	11	*FOLH1*	1387	0.39	0.5	0.8	1.0
rs2290684	19	*DNMT1*	1388	0.47	0.8	0.8	1.0
rs4672768	2	*ATIC*	1385	0.31	0.9	0.8	1.0
rs9306139	21	*SLC19A1*	1386	0.21	0.8	0.8	1.0
rs4144700	11	*FOLH1*	1388	0.38	0.3	0.8	1.0
rs12797853	11	*FOLH1*	1385	0.13	0.7	0.8	1.0
rs8003567	14	*MTHFD1*	1388	0.10	0.8	0.8	1.0
rs11887120	2	*DNMT3A*	1388	0.42	0.5	0.8	1.0
rs11627525	14	*MTHFD1*	1388	0.10	0.3	0.8	1.0
rs2838970	21	*SLC19A1*	1387	0.39	0.9	0.8	1.0
rs4673991	2	*ATIC*	1387	0.31	0.9	0.8	1.0
rs4646383	17	*PEMT*	1387	0.09	0.5	0.8	1.0
rs1801131	1	*MTHFR*	1322	0.28	0.9	0.8	1.0
rs2183601	21	*SLC19A1*	1388	0.21	1.0	0.8	1.0
rs10420338	19	*DNMT1*	1388	0.47	0.6	0.8	1.0
rs4673993	2	*ATIC*	1388	0.31	0.9	0.8	1.0
rs7279305	21	*SLC19A1*	1388	0.35	1.0	0.8	1.0
rs9621047	22	*TCN2*	1388	0.44	0.2	0.8	1.0
rs11701960	21	*SLC19A1*	1387	0.17	1.0	0.8	1.0
rs1164685	11	*FOLH1*	1385	0.38	0.3	0.8	1.0
rs443394	15	*MTHFS*	1388	0.41	0.3	0.9	1.0
rs4476347	2	*ATIC*	1387	0.25	0.4	0.9	1.0

Supplementary Table S5-6 (cont.) Uncorrected and corrected p-values for the interaction between D4Z4 methylation levels and selected SNPs in the one-carbon metabolism pathway in the Spanish Bladder Cancer/EPICURO Study

dbSNP ID (N=495)	Chromosome	Gene	N	MAF	*P* HWE	*P* LRT	Corrected *P* LRT
rs2150460	21	*SLC19A1*	1388	0.21	0.9	0.9	1.0
rs2866358	11	*FOLH1*	1372	0.38	0.2	0.9	1.0
rs6445606	3	*CHDH*	1388	0.29	0.2	0.9	1.0
rs4987173	6	*GNMT*	1388	0.51	0.8	0.9	1.0
rs4778721	15	*MTHFS*	1388	0.23	0.2	0.9	1.0
rs2877078	21	*SLC19A1*	1376	0.39	0.7	0.9	1.0
rs4778719	15	*MTHFS*	1388	0.23	0.2	0.9	1.0
rs16971252	15	*MTHFS*	1388	0.06	0.3	0.9	1.0
rs7219568	17	*PEMT*	1388	0.05	1.0	0.9	1.0
rs582172	15	*MTHFS*	1388	0.41	0.6	0.9	1.0
rs13002567	2	*DNMT3A*	1388	0.28	0.5	0.9	1.0
rs9462856	6	*GNMT*	1388	0.41	0.9	0.9	1.0
rs1473406	15	*MTHFS*	1386	0.15	0.9	0.9	1.0
rs2838977	21	*SLC19A1*	1386	0.39	0.9	0.9	1.0
rs1809986	11	*FOLH1*	1388	0.37	1.0	0.9	1.0
rs2228611	19	*DNMT1*	1388	0.47	0.8	0.9	1.0
rs7120743	11	*FOLH1*	1371	0.37	0.8	0.9	1.0
rs731991	22	*TCN2*	1343	0.48	0.1	0.9	1.0
rs8003379	14	*MTHFD1*	1387	0.24	0.4	0.9	1.0
rs4820888	22	*TCN2*	1387	0.47	0.1	0.9	1.0
rs376863	15	*MTHFS*	1366	0.50	0.9	0.9	1.0
rs1023159	21	*SLC19A1*	1386	0.42	0.4	0.9	1.0
rs7586294	2	*DNMT3A*	1387	0.47	0.5	0.9	1.0
rs4779148	15	*MTHFS*	1388	0.11	0.2	0.9	1.0
rs2241807	3	*CHDH*	1388	0.43	0.7	0.9	1.0
rs13427202	2	*DNMT3A*	1387	0.47	0.6	0.9	1.0
rs8129445	21	*SLC19A1*	1388	0.32	0.9	0.9	1.0
rs1801394	5	*MTRR*	1398	0.49	0.8	0.9	1.0
rs6722613	2	*DNMT3A*	1388	0.40	1.0	0.9	1.0
rs1127717	3	*ALDH1L1*	1388	0.24	0.6	1.0	1.0
rs2236222	14	*MTHFD1*	1388	0.10	0.5	1.0	1.0
rs759920	19	*DNMT1*	1388	0.47	0.7	1.0	1.0
rs5753231	22	*TCN2*	1387	0.16	0.8	1.0	1.0
rs767138	21	*SLC19A1*	1385	0.40	0.6	1.0	1.0
rs1604503	15	*MTHFS*	1388	0.14	0.4	1.0	1.0
rs3783728	14	*MTHFD1*	1388	0.08	1.0	1.0	1.0
rs4673981	2	*ATIC*	1388	0.39	0.8	1.0	1.0
rs1808119	2	*ATIC*	1388	0.20	1.0	1.0	1.0
rs11871738	17	*PEMT*	1388	0.38	0.4	1.0	1.0

Supplementary Table S5-6 (cont.) Uncorrected and corrected p-values for the interaction between D4Z4 methylation levels and selected SNPs in the one-carbon metabolism pathway in the Spanish Bladder Cancer/EPICURO Study

dbSNP ID (N=495)	Chromosome	Gene	N	MAF	*P* HWE	*P* LRT	Corrected *P* LRT
rs12997662	2	*ATIC*	1387	0.33	0.7	1.0	1.0
rs13036246	2	*DNMT3A*	1387	0.49	0.4	1.0	1.0
rs6058894	20	*DNMT3B*	1387	0.08	0.5	1.0	1.0
rs6058896	20	*DNMT3B*	1388	0.08	0.8	1.0	1.0
rs16971231	15	*MTHFS*	1385	0.05	0.08	1.0	1.0
rs11040106	11	*FOLH1*	1379	0.36	0.6	1.0	1.0
rs9836592	3	*CHDH*	1388	0.31	0.3	1.0	1.0
rs8018032	14	*MTHFD1*	1388	0.45	0.7	1.0	1.0
rs7276295	21	*SLC19A1*	1388	0.06	0.3	1.0	1.0
rs17279753	15	*MTHFS*	1388	0.20	0.5	1.0	1.0
rs7085104	10	*AS3MT*	1388	0.39	1.0	1.0	1.0
rs2301955	22	*TCN2*	1386	0.43	0.3	1.0	1.0
rs2987969	14	*MTHFD1*	1387	0.45	0.7	1.0	1.0
rs17751556	14	*MTHFD1*	1388	0.08	1.0	1.0	1.0
rs1550117	2	*DNMT3A*	1387	0.08	1.0	1.0	1.0
rs17279885	15	*MTHFS*	1388	0.21	0.7	1.0	1.0
rs5749135	22	*TCN2*	1388	0.43	0.3	1.0	1.0
rs11629135	14	*MTHFD1*	1388	0.10	0.7	1.0	1.0

Supplementary Table S5-6 (cont.) Uncorrected and corrected p-values for the interaction between D4Z4 methylation levels and selected SNPs in the one-carbon metabolism pathway in the Spanish Bladder Cancer/EPICURO Study

SNPs modeled in recessive mode of inheritance							
dbSNP ID (N=375)	Chromosome	Gene	N	MAF	*P* HWE	*P* LRT	Corrected *P* LRT
rs1256142	14	*MTHFD1*	1388	0.44	0.07	0.0004	0.1
rs7124266	11	*FOLH1*	1388	0.30	0.6	0.003	0.5
rs2834235	21	*GART*	1388	0.38	0.7	0.003	0.5
rs2236224	14	*MTHFD1*	1388	0.36	0.1	0.005	0.7
rs2236225	14	*MTHFD1*	1387	0.43	0.2	0.006	0.8
rs3862342	11	*FOLH1*	1386	0.28	0.8	0.007	0.8
rs2838965	21	*SLC19A1*	1384	0.42	0.05	0.008	0.8
rs8041943	15	*MTHFS*	1385	0.42	0.6	0.009	0.9
rs2154583	21	*GART*	1384	0.39	0.7	0.01	0.9
rs6517178	21	*GART*	1388	0.39	0.7	0.01	0.9
rs3893384	15	*MTHFS*	1388	0.42	0.5	0.02	1.0
rs6495441	15	*MTHFS*	1388	0.24	0.3	0.03	1.0
rs1880580	15	*MTHFS*	1388	0.30	0.09	0.03	1.0
rs4779140	15	*MTHFS*	1387	0.48	0.6	0.03	1.0
rs1081235	15	*MTHFS*	1388	0.19	0.3	0.04	1.0
rs7563206	2	*ATIC*	1387	0.47	0.6	0.04	1.0
rs12910340	15	*MTHFS*	1388	0.42	1.0	0.04	1.0
rs1880586	2	*ATIC*	1387	0.47	0.6	0.04	1.0
rs2424932	20	*DNMT3B*	1387	0.43	0.5	0.04	1.0
rs10839296	11	*FOLH1*	1371	0.25	0.8	0.04	1.0
rs7107178	11	*FOLH1*	1387	0.25	1.0	0.04	1.0
rs663649	1	*CTH*	1388	0.31	0.9	0.05	1.0
rs4646404	17	*PEMT*	1383	0.36	0.3	0.05	1.0
rs7560488	2	*DNMT3A*	1333	0.47	0.2	0.05	1.0
rs4817579	21	*GART*	1388	0.35	1.0	0.05	1.0
rs473334	1	*CTH*	1388	0.31	0.9	0.05	1.0
rs7111711	11	*FOLH1*	1388	0.25	0.9	0.05	1.0
rs4817577	21	*GART*	1388	0.34	0.9	0.06	1.0
rs7283354	21	*GART*	1388	0.34	0.9	0.07	1.0
rs7111215	11	*FOLH1*	1368	0.40	0.2	0.08	1.0
rs1801131	1	*MTHFR*	1322	0.28	0.9	0.08	1.0
rs3821353	2	*ATIC*	1388	0.21	0.4	0.08	1.0
rs4911263	20	*DNMT3B*	1388	0.32	1.0	0.08	1.0
rs1917311	11	*FOLH1*	1353	0.40	0.4	0.08	1.0
rs9332	5	*MTRR*	1388	0.12	0.2	0.09	1.0
rs663465	1	*CTH*	1387	0.41	0.4	0.09	1.0
rs1164685	11	*FOLH1*	1385	0.38	0.3	0.1	1.0
rs16853782	2	*ATIC*	1388	0.21	0.7	0.1	1.0

Supplementary Table S5-6 (cont.) Uncorrected and corrected p-values for the interaction between D4Z4 methylation levels and selected SNPs in the one-carbon metabolism pathway in the Spanish Bladder Cancer/EPICURO Study

dbSNP ID (N=375)	Chromosome	Gene	N	MAF	*P* HWE	*P* LRT	Corrected *P* LRT
rs1074390	15	*MTHFS*	1388	0.37	0.6	0.1	1.0
rs2733103	15	*MTHFS*	1387	0.15	0.2	0.1	1.0
rs4987173	6	*GNMT*	1388	0.51	0.8	0.1	1.0
rs2586154	15	*MTHFS*	1388	0.15	0.5	0.1	1.0
rs12903985	15	*MTHFS*	1388	0.28	0.4	0.1	1.0
rs6058869	20	*DNMT3B*	1388	0.33	0.7	0.1	1.0
rs7220132	17	*PEMT*	1388	0.29	0.7	0.1	1.0
rs2183601	21	*SLC19A1*	1388	0.21	1.0	0.1	1.0
rs2150460	21	*SLC19A1*	1388	0.21	0.9	0.1	1.0
rs9976878	21	*SLC19A1*	1387	0.21	0.8	0.1	1.0
rs9306139	21	*SLC19A1*	1386	0.21	0.8	0.1	1.0
rs11191457	10	*AS3MT*	1385	0.23	0.4	0.1	1.0
rs7085854	10	*AS3MT*	1387	0.23	0.6	0.1	1.0
rs4479310	17	*PEMT*	1388	0.29	0.6	0.1	1.0
rs7587636	2	*DNMT3A*	1388	0.45	0.1	0.1	1.0
rs11085720	19	*DNMT1*	1388	0.42	0.5	0.1	1.0
rs10420338	19	*DNMT1*	1388	0.47	0.6	0.1	1.0
rs2586182	15	*MTHFS*	1388	0.15	0.4	0.1	1.0
rs759920	19	*DNMT1*	1388	0.47	0.7	0.1	1.0
rs2838973	21	*SLC19A1*	1388	0.21	1.0	0.1	1.0
rs585800	5	*BHMT*	1388	0.26	0.05	0.1	1.0
rs4434082	21	*SLC19A1*	1387	0.21	0.9	0.1	1.0
rs4441015	11	*FOLH1*	1353	0.15	0.5	0.1	1.0
rs588458	11	*FOLH1*	1367	0.38	0.3	0.1	1.0
rs16999714	19	*DNMT1*	1386	0.21	0.1	0.1	1.0
rs2290684	19	*DNMT1*	1388	0.47	0.8	0.1	1.0
rs4144700	11	*FOLH1*	1388	0.38	0.3	0.1	1.0
rs7946	17	*PEMT*	1387	0.29	0.4	0.1	1.0
rs865646	5	*DHFR*	1340	0.34	0.4	0.1	1.0
rs7174668	15	*MTHFS*	1388	0.20	0.2	0.1	1.0
rs13401241	2	*DNMT3A*	1388	0.45	0.1	0.2	1.0
rs2228611	19	*DNMT1*	1388	0.47	0.8	0.2	1.0
rs2115536	15	*MTHFS*	1388	0.50	0.05	0.2	1.0
rs282792	15	*MTHFS*	1388	0.39	0.6	0.2	1.0
rs2865908	11	*FOLH1*	1388	0.19	0.9	0.2	1.0
rs2866358	11	*FOLH1*	1372	0.38	0.2	0.2	1.0
rs3772078	2	*ATIC*	1388	0.21	0.6	0.2	1.0
rs282795	15	*MTHFS*	1387	0.32	0.7	0.2	1.0
rs10418	22	*TCN2*	1367	0.21	0.7	0.2	1.0
rs8074074	17	*PEMT*	1386	0.29	0.8	0.2	1.0

Supplementary Table S5-6 (cont.) Uncorrected and corrected p-values for the interaction between D4Z4 methylation levels and selected SNPs in the one-carbon metabolism pathway in the Spanish Bladder Cancer/EPICURO Study

dbSNP ID (N=375)	Chromosome	Gene	N	MAF	*P* HWE	*P* LRT	Corrected *P* LRT
rs11040421	11	*FOLH1*	1388	0.15	0.6	0.2	1.0
rs3760188	17	*PEMT*	1388	0.46	0.6	0.2	1.0
rs2409495	21	*GART*	1388	0.19	0.05	0.2	1.0
rs8074191	17	*PEMT*	1372	0.28	0.9	0.2	1.0
rs204942	15	*MTHFS*	1388	0.20	0.1	0.2	1.0
NA	1	*MTHFR_02_2_i*	1388	0.40	0.5	0.2	1.0
rs1127717	3	*ALDH1L1*	1388	0.24	0.6	0.2	1.0
rs1801133	1	*MTHFR*	1388	0.40	0.5	0.2	1.0
rs10519256	15	*MTHFS*	1388	0.11	0.1	0.2	1.0
rs1888530	21	*SLC19A1*	1358	0.47	0.07	0.2	1.0
rs4672768	2	*ATIC*	1385	0.31	0.9	0.2	1.0
rs2115540	15	*MTHFS*	1387	0.50	0.05	0.2	1.0
rs3755817	3	*CHDH*	1387	0.30	0.5	0.2	1.0
rs6058893	20	*DNMT3B*	1388	0.33	0.2	0.2	1.0
rs4646344	17	*PEMT*	1388	0.47	0.7	0.2	1.0
rs2838958	21	*SLC19A1*	1385	0.45	0.7	0.2	1.0
rs8659	5	*MTRR*	1386	0.35	0.3	0.2	1.0
rs3785499	17	*PEMT*	1388	0.47	0.8	0.2	1.0
rs9835128	3	*CHDH*	1387	0.17	0.4	0.2	1.0
rs1847638	11	*FOLH1*	1335	0.21	1.0	0.2	1.0
rs10769558	11	*FOLH1*	1388	0.21	0.5	0.2	1.0
rs4094478	11	*FOLH1*	1365	0.20	0.7	0.2	1.0
rs4673993	2	*ATIC*	1388	0.31	0.9	0.2	1.0
rs11158540	14	*MTHFD1*	1388	0.34	0.9	0.2	1.0
rs11677670	2	*DNMT3A*	1382	0.17	0.5	0.2	1.0
rs10839210	11	*FOLH1*	1386	0.21	0.5	0.2	1.0
rs6706415	2	*ATIC*	1388	0.30	0.4	0.2	1.0
rs2733106	15	*MTHFS*	1383	0.15	0.5	0.2	1.0
rs2586153	15	*MTHFS*	1374	0.15	0.4	0.2	1.0
rs7581217	2	*DNMT3A*	1388	0.39	0.2	0.2	1.0
rs2696935	11	*FOLH1*	1388	0.21	0.4	0.2	1.0
rs2696923	11	*FOLH1*	1388	0.21	0.3	0.2	1.0
rs4673991	2	*ATIC*	1387	0.31	0.9	0.2	1.0
rs6722613	2	*DNMT3A*	1388	0.40	1.0	0.2	1.0
rs11871738	17	*PEMT*	1388	0.38	0.4	0.3	1.0
rs2267163	22	*TCN2*	1386	0.43	0.2	0.3	1.0
rs1983462	2	*ATIC*	1388	0.30	0.3	0.3	1.0
rs1801198	22	*TCN2*	1387	0.43	0.3	0.3	1.0
rs12373907	21	*SLC19A1*	1387	0.38	0.4	0.3	1.0
rs3783	17	*SHMT1*	1398	0.26	0.6	0.3	1.0

Supplementary Table S5-6 (cont.) Uncorrected and corrected p-values for the interaction between D4Z4 methylation levels and selected SNPs in the one-carbon metabolism pathway in the Spanish Bladder Cancer/EPICURO Study

dbSNP ID (N=375)	Chromosome	Gene	N	MAF	*P* HWE	*P* LRT	Corrected *P* LRT
rs11869600	17	*PEMT*	1386	0.38	0.3	0.3	1.0
rs2275566	1	*MTR*	1388	0.42	0.1	0.3	1.0
rs282776	15	*MTHFS*	1388	0.36	1.0	0.3	1.0
rs7951180	11	*FOLH1*	1369	0.17	0.4	0.3	1.0
rs443394	15	*MTHFS*	1388	0.41	0.3	0.3	1.0
rs12121543	1	*MTHFR*	1388	0.22	0.9	0.3	1.0
rs1046778	10	*AS3MT*	1388	0.33	0.8	0.3	1.0
rs3788190	21	*SLC19A1*	1387	0.47	0.1	0.3	1.0
rs1051296	21	*SLC19A1*	1375	0.47	0.1	0.3	1.0
rs10179873	2	*ATIC*	1388	0.30	0.4	0.3	1.0
rs12997662	2	*ATIC*	1387	0.33	0.7	0.3	1.0
rs648372	11	*FOLH1*	1370	0.17	0.5	0.3	1.0
rs8011839	14	*MTHFD1*	1388	0.18	0.4	0.3	1.0
rs11887120	2	*DNMT3A*	1388	0.42	0.5	0.3	1.0
rs2424908	20	*DNMT3B*	1388	0.17	0.7	0.3	1.0
rs34048824	2	*DNMT3A*	1387	0.50	1.0	0.3	1.0
rs8971	21	*GART*	1386	0.26	0.8	0.3	1.0
rs1051298	21	*SLC19A1*	1387	0.46	0.3	0.3	1.0
rs4924922	17	*PEMT*	1388	0.36	0.3	0.3	1.0
rs4911107	20	*DNMT3B*	1388	0.32	0.6	0.3	1.0
rs770144	15	*MTHFS*	1388	0.20	0.3	0.3	1.0
rs2834231	21	*GART*	1388	0.26	0.7	0.3	1.0
rs1846285	11	*FOLH1*	1385	0.17	0.2	0.3	1.0
rs2834232	21	*GART*	1387	0.26	0.7	0.3	1.0
rs11683424	2	*DNMT3A*	1388	0.12	0.5	0.3	1.0
rs12482346	21	*SLC19A1*	1388	0.47	0.1	0.3	1.0
rs6485991	11	*FOLH1*	1382	0.16	0.6	0.3	1.0
rs897453	17	*PEMT*	1384	0.49	0.6	0.3	1.0
rs4779141	15	*MTHFS*	1386	0.34	0.8	0.4	1.0
rs1805087	1	*MTR*	1388	0.17	0.7	0.4	1.0
rs4646410	17	*PEMT*	1384	0.31	0.9	0.4	1.0
rs515064	1	*CTH*	1388	0.36	0.8	0.4	1.0
rs16853834	2	*ATIC*	1388	0.16	1.0	0.4	1.0
rs685487	15	*MTHFS*	1388	0.37	0.09	0.4	1.0
rs372447	15	*MTHFS*	1388	0.38	0.9	0.4	1.0
rs7253062	19	*DNMT1*	1388	0.39	0.7	0.4	1.0
rs1808119	2	*ATIC*	1388	0.20	1.0	0.4	1.0
rs11040198	11	*FOLH1*	1381	0.21	0.2	0.4	1.0
rs17285431	15	*MTHFS*	1388	0.17	0.1	0.4	1.0
rs5749131	22	*TCN2*	1388	0.43	0.2	0.4	1.0

Supplementary Table S5-6 (cont.) Uncorrected and corrected p-values for the interaction between D4Z4 methylation levels and selected SNPs in the one-carbon metabolism pathway in the Spanish Bladder Cancer/EPICURO Study

dbSNP ID (N=375)	Chromosome	Gene	N	MAF	*P* HWE	*P* LRT	Corrected *P* LRT
rs2838956	21	*SLC19A1*	1373	0.44	0.1	0.4	1.0
rs679470	11	*FOLH1*	1387	0.17	0.6	0.4	1.0
rs12148881	15	*MTHFS*	1387	0.27	0.2	0.4	1.0
rs1979277	17	*SHMT1_01_OR*	1398	0.27	0.8	0.4	1.0
NA	1	*MTR*	1389	0.16	0.7	0.4	1.0
rs12438477	15	*MTHFS*	1387	0.36	0.4	0.4	1.0
rs35020344	14	*MTHFD1*	1387	0.47	0.7	0.4	1.0
rs1369703	2	*DNMT3A*	1388	0.45	0.2	0.4	1.0
rs2983733	14	*MTHFD1*	1388	0.45	0.9	0.4	1.0
rs17209637	15	*MTHFS*	1384	0.25	1.0	0.4	1.0
rs282814	15	*MTHFS*	1388	0.21	0.8	0.4	1.0
rs748196	17	*PEMT*	1385	0.42	0.9	0.4	1.0
rs6141813	20	*DNMT3B*	1388	0.13	0.8	0.4	1.0
rs2983736	14	*MTHFD1*	1385	0.45	0.9	0.4	1.0
rs6087990	20	*DNMT3B*	1388	0.33	0.6	0.4	1.0
rs4532960	10	*AS3MT*	1387	0.44	0.3	0.4	1.0
rs2987969	14	*MTHFD1*	1387	0.45	0.7	0.4	1.0
rs12899781	15	*MTHFS*	1383	0.17	0.1	0.4	1.0
rs10748835	10	*AS3MT*	1388	0.44	0.3	0.4	1.0
rs11892429	2	*ATIC*	1388	0.29	0.7	0.4	1.0
rs445263	15	*MTHFS*	1388	0.30	0.9	0.4	1.0
rs8018032	14	*MTHFD1*	1388	0.45	0.7	0.4	1.0
rs11040416	11	*FOLH1*	1388	0.42	0.4	0.4	1.0
rs6711622	2	*DNMT3A*	1388	0.44	1.0	0.5	1.0
rs10460566	2	*DNMT3A*	1388	0.27	0.3	0.5	1.0
rs1465825	2	*DNMT3A*	1387	0.27	0.3	0.5	1.0
rs2987981	14	*MTHFD1*	1388	0.25	0.9	0.5	1.0
rs4819128	21	*SLC19A1*	1388	0.44	0.2	0.5	1.0
rs12416687	10	*AS3MT*	1388	0.28	0.06	0.5	1.0
rs10498034	2	*ATIC*	1388	0.17	1.0	0.5	1.0
rs9890064	17	*PEMT*	1388	0.43	0.4	0.5	1.0
rs12627639	21	*SLC19A1*	1387	0.21	0.5	0.5	1.0
rs1473406	15	*MTHFS*	1386	0.15	0.9	0.5	1.0
rs8003379	14	*MTHFD1*	1387	0.24	0.4	0.5	1.0
rs6760069	2	*ATIC*	1387	0.14	0.5	0.5	1.0
rs12999687	2	*DNMT3A*	1386	0.45	0.8	0.5	1.0
rs9323450	14	*MTHFD1*	1388	0.30	0.8	0.5	1.0
rs4646359	17	*PEMT*	1388	0.45	0.3	0.5	1.0
rs1076991	14	*MTHFD1*	1388	0.45	1.0	0.5	1.0
rs1801394	5	*MTRR*	1398	0.49	0.8	0.5	1.0

Supplementary Table S5-6 (cont.) Uncorrected and corrected p-values for the interaction between D4Z4 methylation levels and selected SNPs in the one-carbon metabolism pathway in the Spanish Bladder Cancer/EPICURO Study

dbSNP ID (N=375)	Chromosome	Gene	N	MAF	*P* HWE	*P* LRT	Corrected *P* LRT
NA	1	*MTHFR_02_OR*	1285	0.39	0.4	0.5	1.0
rs10197559	2	*ATIC*	1380	0.29	0.9	0.5	1.0
rs1809986	11	*FOLH1*	1388	0.37	1.0	0.5	1.0
rs5753231	22	*TCN2*	1387	0.16	0.8	0.5	1.0
rs376863	15	*MTHFS*	1366	0.50	0.9	0.5	1.0
NA	1	*MTR_01_2_i*	1388	0.17	0.7	0.5	1.0
rs4244599	17	*PEMT*	1371	0.46	0.4	0.5	1.0
rs6713377	2	*DNMT3A*	1387	0.47	0.9	0.5	1.0
rs6058883	20	*DNMT3B*	1387	0.39	0.3	0.5	1.0
rs11158538	14	*MTHFD1*	1385	0.45	0.9	0.5	1.0
rs1950902	14	*MTHFD1*	1388	0.14	0.4	0.5	1.0
rs731991	22	*TCN2*	1343	0.48	0.1	0.5	1.0
rs7575625	2	*DNMT3A*	1388	0.47	0.9	0.5	1.0
rs1979276	17	*SHMT1*	1395	0.30	1.0	0.5	1.0
rs6058891	20	*DNMT3B*	1386	0.39	0.2	0.5	1.0
rs6445606	3	*CHDH*	1388	0.29	0.2	0.5	1.0
rs435689	15	*MTHFS*	1388	0.49	0.9	0.5	1.0
rs2424921	20	*DNMT3B*	1388	0.39	0.3	0.5	1.0
rs9836592	3	*CHDH*	1388	0.31	0.3	0.5	1.0
rs7120743	11	*FOLH1*	1371	0.37	0.8	0.5	1.0
rs2424914	20	*DNMT3B*	1388	0.39	0.4	0.5	1.0
rs2288349	19	*DNMT1*	1387	0.39	0.5	0.6	1.0
rs11040106	11	*FOLH1*	1379	0.36	0.6	0.6	1.0
rs8081810	17	*PEMT*	1387	0.21	0.9	0.6	1.0
rs914238	21	*SLC19A1*	1388	0.49	0.4	0.6	1.0
rs910085	20	*DNMT3B*	1387	0.29	0.5	0.6	1.0
rs567754	5	*BHMT*	1387	0.29	0.9	0.6	1.0
rs2162560	19	*DNMT1*	1387	0.39	0.6	0.6	1.0
rs6511677	19	*DNMT1*	1387	0.39	0.6	0.6	1.0
rs2289093	2	*DNMT3A*	1388	0.28	0.2	0.6	1.0
rs1404774	2	*ATIC*	1380	0.21	0.4	0.6	1.0
rs12987326	2	*DNMT3A*	1388	0.37	0.7	0.6	1.0
rs1667627	14	*MTHFD2*	1387	0.46	0.2	0.6	1.0
rs9974061	21	*SLC19A1*	1388	0.18	1.0	0.6	1.0
rs2330183	21	*SLC19A1*	1358	0.44	0.1	0.6	1.0
rs914232	21	*SLC19A1*	1387	0.44	0.2	0.6	1.0
rs7594432	2	*DNMT3A*	1388	0.45	0.3	0.6	1.0
rs2424922	20	*DNMT3B*	1387	0.39	0.2	0.6	1.0
rs7085104	10	*AS3MT*	1388	0.39	1.0	0.6	1.0
rs12898670	15	*MTHFS*	1387	0.32	0.9	0.6	1.0

Supplementary Table S5-6 (cont.) Uncorrected and corrected p-values for the interaction between D4Z4 methylation levels and selected SNPs in the one-carbon metabolism pathway in the Spanish Bladder Cancer/EPICURO Study

dbSNP ID (N=375)	Chromosome	Gene	N	MAF	*P* HWE	*P* LRT	Corrected *P* LRT
rs13427202	2	*DNMT3A*	1387	0.47	0.6	0.6	1.0
rs282778	15	*MTHFS*	1388	0.27	0.8	0.6	1.0
rs202676	11	*FOLH1*	1380	0.17	0.8	0.6	1.0
rs11694842	2	*DNMT3A*	1387	0.28	0.4	0.6	1.0
rs1256112	14	*MTHFD1*	1388	0.40	0.4	0.6	1.0
rs914231	21	*SLC19A1*	1383	0.44	0.2	0.6	1.0
rs750546	17	*PEMT*	1373	0.43	0.5	0.6	1.0
rs1464864	2	*ATIC*	1388	0.30	0.8	0.6	1.0
rs10839295	11	*FOLH1*	1387	0.39	0.5	0.6	1.0
rs10197653	2	*ATIC*	1388	0.29	0.1	0.6	1.0
rs6518253	21	*SLC19A1*	1387	0.46	0.3	0.6	1.0
rs4911108	20	*DNMT3B*	1384	0.29	0.7	0.7	1.0
rs11681447	2	*DNMT3A*	1386	0.28	0.2	0.7	1.0
rs12591436	15	*MTHFS*	1388	0.35	0.6	0.7	1.0
rs17745484	2	*DNMT3A*	1387	0.35	0.8	0.7	1.0
rs9977111	21	*SLC19A1*	1355	0.33	0.07	0.7	1.0
rs8129445	21	*SLC19A1*	1388	0.32	0.9	0.7	1.0
rs4804122	19	*DNMT1*	1388	0.40	0.4	0.7	1.0
rs1059394	18	*TYMS*	1387	0.31	0.6	0.7	1.0
rs234706	21	*CBS*	1388	0.32	0.1	0.7	1.0
rs3788200	21	*SLC19A1*	1388	0.45	0.3	0.7	1.0
rs2305230	3	*ALDH1L1*	1387	0.19	0.7	0.7	1.0
rs699517	18	*TYMS*	1387	0.31	0.6	0.7	1.0
rs1256095	14	*MTHFD1*	1377	0.48	0.8	0.7	1.0
rs12453139	17	*PEMT*	1386	0.26	0.9	0.7	1.0
rs1109859	17	*PEMT*	1367	0.18	0.8	0.7	1.0
rs7605753	2	*DNMT3A*	1388	0.47	0.6	0.7	1.0
rs617219	5	*BHMT*	1387	0.32	0.7	0.7	1.0
rs1256107	14	*MTHFD1*	1387	0.48	0.7	0.7	1.0
rs600671	15	*MTHFS*	1387	0.46	0.1	0.7	1.0
rs4673965	2	*ATIC*	1388	0.40	1.0	0.7	1.0
rs12905663	15	*MTHFS*	1381	0.27	0.2	0.7	1.0
rs1077965	15	*MTHFS*	1387	0.41	0.4	0.7	1.0
rs7586294	2	*DNMT3A*	1387	0.47	0.5	0.7	1.0
rs740234	22	*TCN2*	1388	0.23	0.8	0.7	1.0
rs6749992	2	*DNMT3A*	1388	0.47	0.8	0.7	1.0
rs11656215	17	*PEMT*	1388	0.45	0.4	0.7	1.0
rs8101626	19	*DNMT1*	1388	0.39	0.7	0.7	1.0
rs12898642	15	*MTHFS*	1387	0.43	0.2	0.7	1.0
rs7215833	17	*PEMT*	1388	0.35	0.5	0.7	1.0

Supplementary Table S5-6 (cont.) Uncorrected and corrected p-values for the interaction between D4Z4 methylation levels and selected SNPs in the one-carbon metabolism pathway in the Spanish Bladder Cancer/EPICURO Study

dbSNP ID (N=375)	Chromosome	Gene	N	MAF	*P* HWE	*P* LRT	Corrected *P* LRT
rs4673981	2	*ATIC*	1388	0.39	0.8	0.7	1.0
rs4819130	21	*SLC19A1*	1384	0.45	0.2	0.7	1.0
rs898436	15	*MTHFS*	1384	0.46	0.1	0.7	1.0
rs1888533	21	*SLC19A1*	1388	0.48	0.3	0.7	1.0
rs3740392	10	*AS3MT*	1385	0.29	0.1	0.7	1.0
rs17291414	19	*DNMT1*	1388	0.27	0.5	0.7	1.0
rs282772	15	*MTHFS*	1388	0.13	0.3	0.7	1.0
rs4818789	21	*SLC19A1*	1388	0.25	0.5	0.7	1.0
rs11701960	21	*SLC19A1*	1387	0.17	1.0	0.7	1.0
rs3177999	21	*SLC19A1*	1380	0.45	0.2	0.7	1.0
rs10498036	2	*ATIC*	1388	0.40	0.4	0.7	1.0
rs1023159	21	*SLC19A1*	1386	0.42	0.4	0.7	1.0
rs378057	15	*MTHFS*	1387	0.14	1.0	0.7	1.0
rs2424928	20	*DNMT3B*	1388	0.39	0.2	0.7	1.0
rs2301955	22	*TCN2*	1386	0.43	0.3	0.7	1.0
rs2424913	20	*DNMT3B*	1387	0.37	0.2	0.8	1.0
rs1051266	21	*SLC19A1*	1388	0.45	0.2	0.8	1.0
rs4531931	2	*ATIC*	1387	0.31	0.5	0.8	1.0
rs9462856	6	*GNMT*	1388	0.41	0.9	0.8	1.0
rs9789571	2	*ATIC*	1388	0.42	1.0	0.8	1.0
rs7604984	2	*ATIC*	1388	0.40	0.4	0.8	1.0
rs17824591	14	*MTHFD1*	1386	0.23	0.2	0.8	1.0
rs749130	2	*DNMT3A*	1388	0.44	0.6	0.8	1.0
rs5749135	22	*TCN2*	1388	0.43	0.3	0.8	1.0
rs1814175	11	*FOLH1*	1384	0.39	0.4	0.8	1.0
rs1802059	5	*MTRR*	1387	0.37	0.4	0.8	1.0
rs2424906	20	*DNMT3B*	1388	0.38	0.2	0.8	1.0
rs767138	21	*SLC19A1*	1385	0.40	0.6	0.8	1.0
rs8129350	21	*SLC19A1*	1387	0.35	0.9	0.8	1.0
rs166868	15	*MTHFS*	1387	0.36	1.0	0.8	1.0
rs8128676	21	*SLC19A1*	1350	0.22	1.0	0.8	1.0
rs3862350	11	*FOLH1*	1360	0.39	0.2	0.8	1.0
rs12614943	2	*ATIC*	1388	0.28	1.0	0.8	1.0
rs6058897	20	*DNMT3B*	1388	0.43	0.2	0.8	1.0
rs7583409	2	*DNMT3A*	1387	0.35	0.4	0.8	1.0
rs17284990	15	*MTHFS*	1388	0.22	0.5	0.8	1.0
rs4819138	21	*SLC19A1*	1388	0.39	0.8	0.8	1.0
rs4646385	17	*PEMT*	1388	0.43	0.5	0.8	1.0
rs7177659	15	*MTHFS*	1387	0.50	0.08	0.8	1.0
rs559062	1	*CTH*	1388	0.22	0.6	0.8	1.0

Supplementary Table S5-6 (cont.) Uncorrected and corrected p-values for the interaction between D4Z4 methylation levels and selected SNPs in the one-carbon metabolism pathway in the Spanish Bladder Cancer/EPICURO Study

dbSNP ID (N=375)	Chromosome	Gene	N	MAF	*P* HWE	*P* LRT	Corrected *P* LRT
rs2124344	17	*PEMT*	1387	0.36	0.7	0.8	1.0
rs202712	11	*FOLH1*	1387	0.23	0.7	0.8	1.0
rs8128050	21	*SLC19A1*	1385	0.35	0.9	0.9	1.0
rs734693	2	*DNMT3A*	1387	0.29	0.2	0.9	1.0
rs2838970	21	*SLC19A1*	1387	0.39	0.9	0.9	1.0
rs7279305	21	*SLC19A1*	1388	0.35	1.0	0.9	1.0
rs1081231	15	*MTHFS*	1387	0.18	0.8	0.9	1.0
rs16971450	15	*MTHFS*	1387	0.16	0.4	0.9	1.0
rs13036246	2	*DNMT3A*	1387	0.49	0.4	0.9	1.0
rs4779165	15	*MTHFS*	1387	0.16	0.4	0.9	1.0
rs7177027	15	*MTHFS*	1388	0.24	0.08	0.9	1.0
rs4646340	17	*PEMT*	1388	0.36	0.5	0.9	1.0
rs6586282	21	*CBS*	1387	0.17	0.2	0.9	1.0
rs2035027	15	*MTHFS*	1388	0.16	0.5	0.9	1.0
rs1380642	15	*MTHFS*	1388	0.18	0.3	0.9	1.0
rs2281603	14	*MTHFD1*	1388	0.19	0.2	0.9	1.0
rs10163099	15	*MTHFS*	1385	0.26	0.1	0.9	1.0
rs4393531	15	*MTHFS*	1386	0.48	0.4	0.9	1.0
rs4778721	15	*MTHFS*	1388	0.23	0.2	0.9	1.0
rs11687225	2	*ATIC*	1387	0.40	0.9	0.9	1.0
rs2838961	21	*SLC19A1*	1388	0.34	1.0	0.9	1.0
rs6801605	3	*CHDH*	1388	0.38	0.6	0.9	1.0
rs4646341	17	*PEMT*	1385	0.36	0.5	0.9	1.0
rs11855092	15	*MTHFS*	1388	0.23	0.07	0.9	1.0
rs4778719	15	*MTHFS*	1388	0.23	0.2	0.9	1.0
rs202700	11	*FOLH1*	1337	0.23	0.8	0.9	1.0
rs4646350	17	*PEMT*	1388	0.36	0.6	0.9	1.0
rs944422	21	*SLC19A1*	1384	0.35	0.9	0.9	1.0
rs2241807	3	*CHDH*	1388	0.43	0.7	0.9	1.0
rs2790	18	*TYMS*	1384	0.20	0.7	0.9	1.0
rs10948059	6	*GNMT*	1370	0.48	0.8	0.9	1.0
rs7604425	2	*ATIC*	1388	0.35	0.7	0.9	1.0
rs11627387	14	*MTHFD1*	1388	0.29	0.9	0.9	1.0
rs10165919	2	*ATIC*	1387	0.35	0.6	0.9	1.0
rs6087988	20	*DNMT3B*	1388	0.20	0.6	0.9	1.0
rs9621047	22	*TCN2*	1388	0.44	0.2	0.9	1.0
rs2838977	21	*SLC19A1*	1386	0.39	0.9	0.9	1.0
rs4610054	2	*ATIC*	1383	0.38	1.0	0.9	1.0
rs853858	20	*DNMT3B*	1386	0.37	0.2	0.9	1.0
rs10839239	11	*FOLH1*	1386	0.23	0.6	0.9	1.0

Supplementary Table S5-6 (cont.) Uncorrected and corrected p-values for the interaction between D4Z4 methylation levels and selected SNPs in the one-carbon metabolism pathway in the Spanish Bladder Cancer/EPICURO Study

dbSNP ID (N=375)	Chromosome	Gene	N	MAF	*P* HWE	*P* LRT	Corrected *P* LRT
rs660439	11	*FOLH1*	1384	0.23	0.7	0.9	1.0
rs17279753	15	*MTHFS*	1388	0.20	0.5	0.9	1.0
rs4820888	22	*TCN2*	1387	0.47	0.1	1.0	1.0
rs1917321	11	*FOLH1*	1380	0.50	0.2	1.0	1.0
rs2289195	2	*DNMT3A*	1387	0.44	0.2	1.0	1.0
rs1650697	5	*DHFR*	1385	0.23	0.1	1.0	1.0
rs4476347	2	*ATIC*	1387	0.25	0.4	1.0	1.0
rs2877078	21	*SLC19A1*	1376	0.39	0.7	1.0	1.0
rs6495446	15	*MTHFS*	1387	0.26	0.1	1.0	1.0
rs13002567	2	*DNMT3A*	1388	0.28	0.5	1.0	1.0
rs8128681	21	*SLC19A1*	1388	0.32	0.9	1.0	1.0
rs582172	15	*MTHFS*	1388	0.41	0.6	1.0	1.0
rs17279885	15	*MTHFS*	1388	0.21	0.7	1.0	1.0
rs2275565	1	*MTR*	1388	0.20	0.6	1.0	1.0
rs11158542	14	*MTHFD1*	1388	0.29	0.8	1.0	1.0
rs1055345	21	*SLC19A1*	1387	0.29	0.3	1.0	1.0
rs6573559	14	*MTHFD1*	1388	0.29	1.0	1.0	1.0

N, number of subjects; MAF, minor allele frequency; P HWE - Hardy-Weinberg Equilibrium chi-square test p-value; P LRT, likelihood ratio test p-value for interaction; NA - dbSNP identifier not available.

Supplementary Table S5-7 Association between D4Z4 methylation level and urothelial carcinoma of the bladder risk stratified by FGFR3 expression and mutation status in the Spanish Bladder Cancer/EPICURO Study

D4Z4 Methylation	Cases	Controls	OR*	95% CI		P-value
Low FGFR3 expression†						
Low-grade NMIBC						
M1, < 64.4%	42	358	1 Reference			
M2, ≥ 64.4%	55	357	1.43	0.92	2.23	0.1
High-grade NMIBC						
M1, < 64.4%	21	358	1 Reference			
M2, ≥ 64.4%	31	357	1.60	0.88	2.91	0.1
MIBC						
M1, < 64.4%	43	358	1 Reference			
M2, ≥ 64.4%	37	357	0.92	0.57	1.49	0.7
Wild type *FGFR3*‡						
Low-grade NMIBC						
M1, < 64.4%	52	358	1 Reference			
M2, ≥ 64.4%	65	357	1.28	0.85	1.92	0.2
High-grade NMIBC						
M1, < 64.4%	40	358	1 Reference			
M2, ≥ 64.4%	47	357	1.29	0.81	2.04	0.3
MIBC						
M1, < 64.4%	54	358	1 Reference			
M2, ≥ 64.4%	45	357	0.88	0.57	1.37	0.6
Mutant *FGFR3*§						
Low-grade NMIBC						
M1, < 64.4%	74	358	1 Reference			
M2, ≥ 64.4%	92	357	1.35	0.95	1.93	0.09
High-grade NMIBC						
M1, < 64.4%	18	333	1 Reference			
M2, ≥ 64.4%	15	329	0.90	0.44	1.86	0.8
MIBC						
M1, < 64.4%	4	358	1 Reference			
M2, ≥ 64.40%	7	357	1.87	0.53	6.66	0.3

M1, D4Z4 methylation level less than the median; M2, D4Z4 methylation level greater than or equal to the median.

*Adjusted for age, gender, region and smoking status (never, occasional, former, and current).

†P value for heterogeneity: low- vs high-grade NMIBC = 0.9; low-grade NMIBC vs MIBC = 0.1; high-grade NMIBC vs MIBC=0.2.

‡P value for heterogeneity: low- vs high-grade NMIBC = 0.9; low-grade NMIBC vs MIBC = 0.1; high-grade NMIBC vs MIBC=0.2.

§P value for heterogeneity: low- vs high-grade NMIBC = 0.3; low-grade NMIBC vs MIBC = 0.6; high-grade NMIBC vs MIBC=0.3.

Supplementary Table S5-8 Comparisons of controls and cases with and without D4Z4 methylation data by main characteristics in the Spanish Bladder Cancer/EPICURO Study

Characteristic	Controls			Cases		
	With D4Z4 data	Without D4Z4 data	P-value*	With D4Z4 data	Without D4Z4 data	P-value*
Age in years, median (IQR)	66 (13)	68 (13)	0.002	68 (13)	68 (13)	0.7
Gender						
Male	642 (89.4)	463 (83.7)	0.003	617 (87.3)	450 (87.9)	0.7
Female	76 (10.6)	90 (16.3)		90 (12.7)	62 (12.1)	
Region						
Barcelona	130 (18.1)	117 (21.2)	0.05	128 (18.1)	101 (19.7)	0.6
Valles	117 (16.3)	73 (13.2)		113 (16.0)	75 (14.7)	
Elche	58 (8.1)	26 (4.7)		55 (7.8)	33 (6.5)	
Tenerife	122 (17.0)	104 (18.8)		120 (17.0)	99 (19.3)	
Asturias	291 (40.5)	233 (42.1)		291 (41.1)	204 (39.8)	
Smoking status†						
Never	206 (28.8)	161 (29.5)	0.4	102 (14.6)	64 (12.5)	0.8
Occasional	53 (7.4)	44 (8.1)		30 (4.3)	22 (4.3)	
Former	261 (36.5)	214 (39.3)		266 (38.0)	204 (39.9)	
Current	195 (27.3)	126 (23.1)		303 (43.2)	221 (43.3)	
Total	718	553		707	512	

IQR, interquartile range.

*Mann-Whitney test for age and D4Z4 methylation levels; Chi-square test for categorical variables.

†Eleven controls and seven cases have missing information on smoking status.

SUPPLEMENTARY MATERIALS CHAPTER VI

Supplementary Table S6-1 Characteristics of non-muscle-invasive bladder cancer patients by LINE-1 and D4Z4 methylation in the Spanish Bladder Cancer/EPICURO study

Characteristics	LINE-1 methylation tertile				D4Z4 methylation median		
	T1	T2	T3	*P*	M1	M2	*P*
	N (%)	N (%)	N (%)	Value	N (%)	N (%)	Value
Total	236	235	235		263	263	
Age in years							
Median (IQR)	66.5 (14.5)	68 (13.0)	69 (11.0)	0.01*	68 (14.0)	68 (12.0)	0.2*
Sex							
Male	199 (84.3)	210 (89.4)	204 (86.8)	0.3	225 (85.6)	234 (89.0)	0.2
Female	37 (15.7)	25 (10.6)	31 (13.2)		38 (14.4)	29 (11.0)	
Residential area							
Barcelona	40 (17.0)	49 (20.9)	38 (16.2)	0.3	46 (17.5)	57 (21.7)	0.1
Valles	41 (17.3)	44 (18.7)	30 (12.7)		36 (13.7)	50 (19.0)	
Elche	13 (5.5)	21 (8.9)	15 (6.4)		19 (7.2)	22 (8.4)	
Tenerife	40 (17.0)	34 (14.5)	43 (18.3)		48 (18.3)	34 (12.9)	
Asturias	102 (43.2)	87 (37.0)	109 (46.4)		114 (43.4)	100 (38.0)	
Smoking status							
Never	39 (16.7)	24 (10.3)	33 (14.2)	0.2	43 (16.6)	31 (11.9)	0.4
Occasional	8 (3.4)	11 (4.7)	7 (3.0)		12 (4.6)	12 (4.6)	
Former	86 (36.8)	99 (42.5)	106 (45.3)		96 (37.1)	109 (41.8)	
Current	101 (43.1)	99 (42.5)	87 (37.3)		108 (41.7)	109 (41.8)	
Missing	2	2	2		4	2	
Tobacco type							
Never	39 (17.1)	24 (10.8)	33 (14.5)	0.7	43 (17.2)	31 (12.4)	0.2
Blond tobacco only	16 (7.0)	20 (9.0)	18 (7.9)		18 (7.6)	18 (7.2)	
Black tobacco only	82 (36.0)	76 (34.2)	83 (36.4)		77 (30.8)	101 (40.4)	
Both types	60 (26.3)	64 (28.8)	58 (25.4)		75 (30.0)	67 (26.8)	
Unknown	31 (13.6)	38 (17.1)	36 (15.8)		36 (14.4)	33 (13.2)	
Missing	8	13	7		13	13	

Supplementary Table S6-1 (cont.) Characteristics of non-muscle-invasive bladder cancer patients by LINE-1 and D4Z4 methylation in the Spanish Bladder Cancer/EPICURO study

Characteristics	LINE-1 methylation tertile				D4Z4 methylation median		
	T1	T2	T3	P-	M1	M2	P-
	N (%)	N (%)	N (%)	Value	N (%)	N (%)	Value
Combined stage and grade (T-G)							
Low-risk tumors							
TaGI†	100 (42.4)	103 (43.8)	87 (37.0)	0.4	99 (37.6)	109 (41.4)	0.3
TaGII	77 (32.6)	66 (28.1)	90 (38.3)		81 (30.8)	93 (35.4)	
High-risk tumors							
TaGIII	20 (8.5)	25 (10.6)	22 (9.4)		31 (11.8)	28 (10.7)	
T1GII	5 (2.1)	3 (1.3)	7 (3.0)		6 (2.3)	5 (1.9)	
T1GIII	33 (14.0)	34 (14.5)	28 (11.9)		42 (16.0)	27 (10.3)	
Tis	1 (0.4)	4 (1.7)	1 (0.4)		4 (1.5)	1 (0.4)	
Tumor Multiplicity							
Solitary	165 (73.7)	144 (65.8)	158 (70.5)	0.2	166 (67.8)	173 (69.5)	0.7
Multiple	59 (26.3)	75 (34.2)	66 (29.5)		79 (32.2)	76 (30.5)	
Missing	12	16	11		18	14	
Tumor Size							
≤ 3 cm	135 (57.2)	136 (57.9)	135 (57.5)	0.4	153 (58.2)	146 (55.5)	0.7
> 3 cm	36 (15.3)	25 (10.6)	37 (15.7)		32 (12.2)	38 (14.5)	
Missing	65 (27.5)	74 (31.5)	63 (26.8)		78 (29.7)	79 (30.0)	
Treatment							
TURBT alone	95 (40.3)	101 (43.0)	95 (40.4)	0.5	96 (36.5)	120 (45.6)	0.1
TURBT+BCG	71 (30.1)	73 (31.1)	65 (27.7)		86 (32.7)	81 (30.8)	
TURBT+Intravesical chemotherapy	60 (25.4)	49 (20.8)	57 (24.3)		66 (25.1)	52 (19.8)	
TURBT+BCG + Intravesical chemotherapy	3 (1.3)	3 (1.3)	9 (3.8)		3 (1.1)	5 (1.9)	
Others	7 (2.9)	9 (3.8)	9 (3.8)		12 (4.6)	5 (1.9)	
Follow-up							
Median (months)	76.1				76.2		

IQR, interquartile range; Tis, carcinoma in situ: “flat tumor”, TURBT, transurethral resection of the bladder tumor; BCG, bacillus Calmette-Guérin.

*P value from Kruskal-Wallis test.

†TaGI includes papillary neoplasm of low malignant potential.

Supplementary Table S6-2 Characteristics of muscle-invasive bladder cancer patients by LINE-1 and D4Z4 methylation in the Spanish Bladder Cancer/EPICURO study

Characteristics	LINE-1 methylation tertile				D4Z4 methylation median		
	T1	T2	T3	P-	M1	M2	P-
	N (%)	N (%)	N (%)	Value	N (%)	N (%)	Value
Total	63	63	63		70	69	
Age in years							
Median (IQR)	68 (12.0)	68 (12.0)	69 (12.0)	1.0*	67.5 (9.0)	71 (12.0)	0.03*
Sex							
Male	57 (90.5)	56 (88.9)	54 (85.7)	0.7	58 (82.9)	65 (94.2)	0.04
Female	6 (9.5)	7 (11.1)	9 (14.3)		12 (17.1)	4 (5.8)	
Residential area							
Barcelona	16 (25.4)	5 (8.0)	7 (11.1)	0.06	12 (17.1)	8 (11.6)	0.3
Valles	10 (15.9)	14 (22.2)	9 (14.3)		9 (12.9)	18 (26.1)	
Elche	3 (4.7)	8 (12.7)	3 (4.7)		4 (5.7)	6 (8.7)	
Tenerife	11 (17.5)	14 (22.2)	12 (19.1)		13 (18.6)	12 (17.4)	
Asturias	23 (36.5)	22 (34.9)	32 (50.8)		32 (45.7)	25 (36.2)	
Smoking status							
Never	7 (11.1)	8 (12.7)	12 (19.5)	0.9	9 (12.9)	9 (13.0)	0.8
Occasional	2 (3.2)	3 (4.8)	2 (3.2)		2 (2.8)	4 (5.8)	
Former	23 (36.5)	20 (31.8)	22 (34.9)		22 (31.4)	24 (34.8)	
Current	31 (49.2)	32 (50.8)	27 (42.9)		37 (52.9)	32 (46.4)	
Tobacco type							
Never	7 (11.5)	8 (13.3)	12 (19.7)	0.1	9 (13.2)	9 (13.9)	0.9
Blond tobacco only	2 (3.3)	8 (13.3)	2 (3.3)		6 (8.8)	4 (6.1)	
Black tobacco only	21 (34.4)	23 (38.3)	26 (42.6)		28 (41.2)	26 (40.0)	
Both types	16 (26.2)	14 (23.3)	11 (18.0)		15 (22.1)	14 (21.5)	
Unknown	15 (24.6)	7 (11.7)	10 (16.4)		10 (14.7)	12 (18.5)	
Missing	2	3	2		2	4	

Supplementary Table S6-2 (cont.) Characteristics of muscle-invasive bladder cancer patients by LINE-1 and D4Z4 methylation in the Spanish Bladder Cancer/EPICURO study

Characteristics	LINE-1 methylation tertile				D4Z4 methylation median		
	T1	T2	T3	P-	M1	M2	P-
	N (%)	N (%)	N (%)	Value	N (%)	N (%)	Value
Tumor stage (T)							
T2	34 (54.0)	34 (54.0)	34 (54.0)	0.7	41 (58.6)	34 (49.3)	0.4
T3	16 (25.4)	11 (17.4)	15 (23.8)		13 (18.6)	19 (27.5)	
T4	13 (20.6)	18 (28.6)	14 (22.2)		16 (22.8)	16 (23.2)	
Regional lymph node (N)							
No lymph node metastases (N0)	39 (61.9)	48 (76.2)	39 (61.9)	0.4	52 (74.3)	43 (62.2)	0.2
Lymph node metastases (N1-N3)	14 (22.2)	9 (14.3)	16 (25.4)		12 (17.1)	13 (18.8)	
Unknown nodal metastases (Nx)	10 (15.9)	6 (9.52)	8 (12.7)		6 (8.6)	13 (18.8)	
Metastasis (M)							
No metastases (M0)	48 (76.2)	56 (88.9)	46 (73.0)	0.09	60 (85.7)	53 (76.8)	0.3
Metastases (M1)	7 (11.1)	3 (4.8)	12 (19.1)		6 (8.6)	7 (10.1)	
Unknown (Mx)	8 (12.7)	4 (6.3)	5 (7.9)		4 (5.7)	9 (13.1)	
Treatment							
Cystectomy	19 (30.2)	27 (42.8)	21 (33.3)	0.6	25 (35.7)	23 (33.3)	0.2
Cystectomy + Systemic chemotherapy	10 (15.9)	9 (14.3)	7 (11.1)		9 (12.9)	10 (14.5)	
Systemic chemotherapy alone	7 (11.1)	5 (7.9)	6 (9.5)		8 (11.4)	3 (4.4)	
RdT+Systemic chemotherapy	5 (7.9)	7 (11.1)	6 (9.5)		4 (5.7)	12 (17.4)	
TURBT+BCG/intravesical chemotherapy	8 (12.7)	3 (4.8)	3 (4.8)		5 (7.1)	5 (7.3)	
Others	14 (22.2)	12 (19.1)	20 (31.8)		19 (27.2)	16 (23.2)	
Follow-up							
Median (months)	23.3				25.5		

IQR, interquartile range; RdT, radiotherapy; TURBT, transurethral resection of the bladder tumor; BCG, bacillus Calmette-Guérin;
**P* value Kruskal-Wallis test

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http://dx.doi.org/10.1289/ehp.1206068

Online 03 April 2013

NIH National Institute of Environmental Health Sciences

Genetic and Non-genetic Predictors of LINE-1 Methylation in Leukocyte DNA

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RUNNING TITLE: Predictors of Global DNA Methylation

KEY WORDS: DNA methylation, epigenetics, LINE-1, one-carbon genetic variants, smoking, trace elements

ACKNOWLEDGMENTS: We acknowledge the members of SMT's thesis committee for comments on the manuscript, and the SBC/EPICURO study participants, technicians, field workers, and coordinators. The work was partially supported by the Association for International

Cancer Research (AICR, #09-0780, including a PhD scholarship awarded to SMT); Fondo de Investigaciones Sanitarias, Instituto de Salud Carlos III, MINECO, Spain (00/0745, PI051436, PI061614, PI09-02102, and G03/174); Red Temática de Investigación Cooperativa en Cáncer-RD06/0020-RTICC; USA-NIH-RO1-CA089715; a postdoctoral fellowship awarded to AFSA from the Fundación Científica de la AECC; Fundació Marató TV3; and The Johns Hopkins University Vredenburg Scholarship awarded to ALC. KPC owns KP Cantor Environmental LLC, Silver Spring, MD, USA. The authors declare they have no competing financial interests.

ABSTRACT

BACKGROUND: Altered DNA methylation has been associated with various diseases.

OBJECTIVE: To evaluate the association between levels of methylation in leukocyte DNA at long interspersed nuclear element 1 (LINE-1) and genetic and non-genetic characteristics of 892 control participants from the Spanish Bladder Cancer/EPICURO Study.

METHODS: We determined LINE-1 methylation levels by pyrosequencing. Individual data included demographics, smoking status, nutrient intake, toenail concentrations of 12 trace elements, xenobiotic metabolism gene variants, and 515 polymorphisms among 24 genes in the one-carbon metabolism pathway. To assess the association between LINE-1 methylation levels (percentage of methylated cytosines) and potential determinants, we estimated beta coefficients (β) by robust linear regression.

RESULTS: Females had lower levels of LINE-1 methylation than males (β=-0.7, p-value=0.02). Blond tobacco smokers showed lower methylation than non-smokers (β=-0.7, p-value=0.03). Arsenic toenail concentration was inversely associated with LINE-1 methylation (β=-3.6, p-value=0.003). By contrast, iron (β=0.002, p-value=0.009) and nickel (β=0.02, p-value=0.004) were positively associated with LINE-1 methylation. SNPs in *DNMT3A* (rs7581217-per allele, β=0.3, p-value=0.002), *TCN2* (rs9606756-GG, β=1.9, p-value=0.008; rs4820887-AA, β=4.0, p-value=$4.8x10^{-7}$; rs9621049-TT, β=4.2, p-value=$4.7x10^{-9}$), *AS3MT* (rs7085104-GG, β=0.7, p-value=0.001), *SLC19A1* (rs914238, TC vs. TT: β=0.5 and CC vs. TT: β=-0.3, global p-value=0.0007) and *MTHFS* (rs1380642, CT vs. CC: β=0.3 and TT vs. CC; β=-0.8, global p-value=0.05) were associated with LINE-1 methylation.

CONCLUSIONS: We identified several characteristics, environmental factors, and common genetic variants that predicted DNA methylation among study participants.

INTRODUCTION

DNA methylation plays a fundamental role in regulation of gene expression, genomic imprinting, X-chromosome inactivation, and repression of transposable elements (Jones and Liang 2009). Aberrant DNA methylation has been associated with various cancers, and with developmental, autoimmune, and other chronic diseases (Robertson 2005). Global DNA methylation can be directly quantified by measuring 5-methylcytosine content of the genome, or can be estimated based on methylation of repetitive sequences such as Alu elements or long interspersed nuclear element 1 (LINE-1) (Yang et al. 2004). Age, sex, smoking, and arsenic and lead exposures have been associated with DNA methylation, but findings have been inconsistent among studies (Breitling et al. 2011; El-Maarri et al. 2007; Fraga et al. 2005a; Fuke et al. 2004; Terry et al. 2011). The folate and methionine-dependent one-carbon metabolism pathway could modulate DNA methylation by altering the level of S-adenosylmethionine (SAM), the principal source of methyl groups (Ulrey et al. 2005). Genetic variants might also influence the methylation of CpG loci locally, or might have a global influence on methylation throughout the genome. For example, a single nucleotide polymorphism (SNP) in *TRPC3*-isoform 2 has been reported to regulate the methylation status of its own promoter (Martin-Trujillo et al. 2011), and variants of the methylenetetrahydrofolate reductase gene *(MTHFR)* have been associated with global DNA hypomethylation (Castro et al. 2004; Friso et al. 2002). However, although the determinants of global and site-specific methylation are widely assumed to be likely contributors to health and disease, they are poorly defined at this time.

Assessing the impact of both genetic and non-genetic factors on global DNA methylation may improve our understanding of the molecular pathogenesis of many common diseases. Therefore, we investigated associations of global DNA methylation in LINE-1 from bisulfite-modified

granulocyte DNA with genetic variants and personal, demographic, lifestyle, and environmental characteristics.

METHODS

Study population: The study population, design, and data collection have been previously described (Garcia-Closas et al. 2005). Briefly, participating individuals were controls from the Spanish Bladder Cancer/EPICURO Study who were admitted to hospitals in five regions of Spain for a range of conditions including hernia, fractures, and other non-cancer diseases, and were 20–81 years of age. We collected demographic and exposure information at the hospitals using computer-assisted personal interviews. From a total of 1,271 controls that agreed to participate in the study and were interviewed, 1,056 provided blood for DNA extraction. We excluded twenty-three subjects because of inadequate or poor quality DNA (N=15) or missing smoking status data (N=8). Three subjects with missing data on smoking status were included because they had data on other variables including age, gender, region, and body mass index (BMI). To ensure homogeneity, we also excluded one non-Caucasian individual, leaving 925 individuals with granulocyte DNA for bisulfite modification and pyrosequencing. Pyrosequencing failed in 33 individuals; thus, the final study population for the present analysis included 892 participants. We obtained written informed consent from all participants, and the study was approved by the local Spanish institutional review boards and US National Cancer Institute.

Quantification of LINE-1 methylation: We extracted granulocyte DNA using standard methods (Garcia-Closas et al. 2005). We carried out bisulfite conversion of DNA using the EZ-96 DNA METHYLATION-GOLD^{TM} KIT (Zymo Research, Irvine, CA, USA) according to the

manufacturer's recommendations. We carried out PCR amplification of bisulfite-modified DNA using a set of forward and reverse primers reported previously (Estecio et al. 2007). To quantify the methylation level of each of the first four CpG sites next to the pyrosequencing primer, we performed sequencing of the PCR product by pyrosequencing, using the PyroMarkTM Q24 System (QIAGEN, Valencia, CA, USA) as recommended by the manufacturer. The first four were the CpGs from which we could obtain methylation values of all samples. We extracted the methylation level at each CpG site using the PyroMarkTM Application Software version 2.0.6 (QIAGEN, Valencia, CA, USA), and we expressed the value as the percentage of methylated cytosines over the sum of methylated and unmethylated cytosines. We used the average methylation level of the first four LINE-1 CpG sites as a surrogate marker of the global DNA methylation level. To determine whether changes in blood cell populations affect LINE-1 methylation levels, we analyzed LINE-1 methylation in independently purified granulocyte and lymphocyte samples. The results showed no significant difference in LINE-1 methylation between granulocyte and lymphocyte samples, thus suggesting that variation in the distribution of peripheral blood cell populations among participants would not contribute to variation in global DNA methylation (data not shown). For the present study, we used DNA extracted from granulocyte to quantify DNA methylation. As a quality control measure, we measured LINE-1 methylation in 129 randomly selected duplicate samples and the within-sample coefficient of variation (CV) was 4.0%. In the analysis, we used the average of the duplicates for those samples.

Nutritional assessment: We estimated the usual intake of vitamins B1, B2, B3, B6, and B12, folate, protein, alcohol, fruit and vegetable over the five years before interview using a validated food frequency questionnaire of 127 items (R Garcia-Closas et al. 2007). Micronutrients and

macronutrients included in the present analysis have been suggested as important co-factors and methyl donors in one-carbon metabolism (Stover 2009). We calculated nutrient density variables by dividing the total estimated mass of daily food consumed by the total estimated daily energy intake (μg/day/kcal).

Trace elements: We collected toenail clippings to estimate chronic exposure to trace elements. Sample collection and experimental methods used to measure trace elements level have been reported (Amaral et al. 2012). Briefly, after cleaning and washing the toenails to remove external contaminants, we quantified elements at the Trace Element Analysis Core (Dartmouth College, NH, USA), using inductively coupled plasma-mass spectrometry (Hopkins et al. 2004). We digested the samples with Optima HNO_3 (Fisher Scientific, St. Louis, MO) at 105 °C followed by addition of H_2O_2 and further heating the dilution with deionized water. We recorded gravimetrically all sample preparation steps. As a quality control, each batch of analyses included six standard reference material samples with known trace element content (SRM; GBW 07601, powdered human hair) and six analytic blanks, along with the study samples. In total, we determined concentrations (μg/g) of 12 trace elements (aluminum, arsenic, cadmium, chromium, copper, iron, lead, manganese, nickel, selenium, vanadium, and zinc).

Genotyping: For genotype assays, we extracted DNA from leukocytes as described previously (Garcia-Closas et al. 2005). We determined genotypes at the Core Genotyping Facility of the Division of Cancer Epidemiology and Genetics, National Cancer Institute, MD, USA. We selected for the analysis a total of 515 SNPs in 24 genes involved in the one-carbon metabolism pathway, including DNA methylation and arsenic metabolism (see Supplemental Material, Table S1 for a list of the 24 genes evaluated). We selected these genes because they are critical for the one-carbon metabolism pathway (Lee et al. 2009; Ulrey et al. 2005). Previously, we described in

detail methods of the genotyping process (Garcia-Closas et al. 2005; M Garcia-Closas et al. 2007; Rothman et al. 2010). We genotyped SNPs using Illumina Infinium® Human1M-Duo, Illumina GoldenGate® and TaqMan® assays (see Supplemental Material, Table S2 for a complete list of SNPs according to assay). In addition, we estimated associations between LINE-1 methylation and *GSTM1, GSTT1,* and *NAT2* variants because of their relevance to bladder cancer (Cash et al. 2012). These variants were determined as described in (Garcia-Closas et al. 2005). All genotypes included in the study were in Hardy-Weinberg Equilibrium in the study population (p-value > 0.05) (data not shown).

Statistical analysis: The distribution of LINE-1 methylation levels was slightly bimodal and positively skewed (Supplemental Material, Figure S1). To estimate associations between LINE-1 methylation levels and each of the variables considered, we fitted bivariate robust linear regression models and calculated the corresponding beta coefficients and 95% confidence intervals (95%CI). Characteristics analyzed as continuous variables were age, micronutrient intakes, fruit and vegetable intakes, and toenail concentrations of trace elements. Characteristics analyzed as categorical variables were BMI (<25.0, 25.0-26.99, 27.0-29.99, ≥30.0), smoking status (non-, occasional, former, current smoker), and tobacco type (non-smoker, blond only, black only, blond and black, unknown).

To identify SNPs for detailed assessment we first used the Fisher's exact test to screen SNPs that were significantly associated (p<0.05) with LINE-1 methylation categorized according to tertiles (<56.7%, 56.7–58.6%, and >58.6%) according to codominant mode of inheritance. The 22 SNPs identified for further analyses (listed in Supplemental Material, Table S3) were subsequently modeled according to all modes of inheritance (additive, codominant, dominant and recessive). The mode of inheritance that best predicted LINE-1 methylation is reported in Table 2.

In addition to age and gender, adjusted robust linear regression models for each potential predictor included region, which may be related to diet, micronutrients (Gabriel et al. 2006), and environmental pollution, and smoking status, which may be related to trace elements (Moerman and Potts 2011). We also did a sensitivity analysis without adjusting for smoking status to see if there was a change in the beta estimates. We included all the 515 SNPs in the analysis regardless of linkage disequilibrium. The association between LINE-1 methylation and potential predictors was assessed in the multivariable adjusted model stratifying by gender. Because arsenic and rs7085104 in arsenic (+3 oxidation state) methyltransferase (*AS3MT*), which is involved in arsenic metabolism, were individually associated with LINE-1 methylation, we assessed the presence of effect modification by including a multiplicative interaction term in a model adjusted for age, gender, region and smoking status. Wald test was used to calculate the interaction p-value. We corrected for multiple testing using the Bonferroni's method. We conducted a sensitivity analysis, excluding 42 individuals with CV >4% for LINE-1 methylation in duplicate samples. This exclusion did not result in substantial differences in the beta coefficients; therefore these individuals remained in the analyses (data not shown). All statistical tests were two-sided, and a p-value $\leq$ 0.05 was considered significant. We carried all data analyses out using STATA/SE version 10.1 (StataCorp, College Station, TX, USA).

RESULTS

The characteristics of the 892 participants in the present study and median and mean LINE-1 methylation levels according to each variable of interest are provided in Supplemental Material, Table S4. The majority of the study participants were males (89%) and regular smokers (63.9%), with median age of 66 years [interquartile range (IQR)=13]. The mean LINE-1 methylation level

was 58.9% (SD=5.3%) with minimum and maximum value of 37.9% and 85.7%, respectively. Table 1 shows the association between LINE-1 methylation levels and characteristics of study subjects. In the bivariable robust linear regression analysis, only toenail arsenic and nickel concentrations were significantly associated with LINE-1 methylation. However, in multivariable robust linear regression models adjusted for age, gender, region, and smoking status, the levels of LINE-1 methylation were significantly lower in females than in males (adjusted β = -0.7; 95%CI: -1.2, -0.1, p-value=0.02) and in smokers of blond tobacco only (adjusted β = -0.7; 95%CI: -1.3, -0.08, p-value=0.03) and of both blond and black tobacco (adjusted β = -0.6; 95%CI: -1.1, -0.07, p-value=0.03) compared with nonsmokers. Toenail arsenic concentration also was negatively associated with LINE-1 methylation (adjusted β for a 1-µg/g increase = -3.6; 95%CI: -5.9, -1.2, p-value=0.003). In contrast, LINE-1 levels were positively associated with 1-µg/g increases in toenail concentrations of iron (adjusted β = 0.002; 95%CI: 0.001, 0.004, p-value=0.009) and nickel (adjusted β = 0.02; 95%CI: 0.005, 0.03, p-value=0.004). BMI, B vitamins, folate, total protein, alcohol, fruit and vegetable intake were not significantly associated with LINE-1 methylation regardless of adjustment for covariates (Table 1). Results from the multivariable analyses without adjusting for smoking status done as a sensitivity analysis were not different from the associations above (Supplemental Material, Table S5). If we were to correct for multiple comparisons by Bonferroni's method none of the above would be significant.

Out of the 515 genetic variants assessed, 22 passed a first screening using Fisher's exact test (p-value≤0.05 according to codominant mode of inheritance) (Supplemental Material, Table S3). Of these, seven SNPs in five genes were significantly associated with LINE-1 methylation based on multivariable models adjusted for age, gender, region, and smoking status (Table 2; model-based

estimates for the 15 SNPs that were not significantly associated with LINE-1 methylation are reported in Supplemental Material, Table S6.) Significant positive associations were estimated for *DNMT3A*-rs7581217 (per allele: adjusted β = 0.3; 95%CI: 0.1, 0.6, p-value=0.002)*; TCN2*-rs9621049 (recessive: adjusted β = 4.2; 95%CI: 2.8, 5.7, p-value=$4.7x10^{-9}$), *TCN2*-rs4820887 (recessive: adjusted β = 4.0; 95%CI: 2.5, 5.6, p-value=$4.8x10^{-7}$) and *TCN2*-rs9606756 (recessive: adjusted β = 1.9; 95%CI: 0.5, 3.3, p-value=0.008)*;* and *AS3MT*-rs7085104 (recessive: adjusted β = 0.7; 95%CI: 0.3, 1.2, p-value=0.001). In addition, significant associations under the codominant mode of inheritance (based on global p-values) were estimated for *SLC19A1*-rs914238 (TC vs. TT, β = 0.5; 95%CI: 0.08, 0.8; CC vs. TT, β = -0.3; 95%CI: -0.7, 0.2; global p-value=0.0007) and *MTHFS*-rs1380642 (CT vs. CC, β = 0.3; 95%CI: -0.08, 0.6; TT vs. CC, β = -0.8; 95%CI: -1.6, 0.09; global p-value=0.05). After correcting for multiple testing using the Bonferroni's method, *TCN2*-rs9621049 and *TCN2*-rs4820887 remained significant (p<0.05).

A significant interaction (p-value=0.01) was observed between arsenic and *AS3MT*-rs7085104 on LINE-1 methylation (adjusted β for 1-µg/g increase in As = -4.1; 95%CI: -6.6, -1.7, p-value=0.001 for genotype AA/AG; and adjusted β for 1-µg/g increase in As = 10.2; 95%CI: -3.2, 23.7, p-value=0.1 for genotype GG).

After simultaneously adjusting for age, geographic region, and all factors that were significant predictors of LINE-1 methylation (sex; tobacco type; toenail arsenic, iron and nickel; and the 5 SNPs noted above), associations with sex, arsenic, nickel, iron, *DNMT3A*-rs7581217, *TCN2*-rs9621049, and *MTHFS*-rs1380642 remained significant. The association with blond tobacco was nonsignificant although the direction of the point estimate remained unchanged. The association between rs9606756, rs4820887 and LINE-1 methylation become nonsignifcant

(Supplemental Material, Table S7). This might be due to reduced sample size in the simultaneously adjusted model due to missing data.

DISCUSSION

In the present study, we took a comprehensive approach to assess associations of genetic and non-genetic factors with LINE-1 methylation in a group of individuals aged 20-81 years. Lower levels of LINE-1 methylation were found among females compared with males, and among smokers of blond tobacco compared with non-smokers. In addition, toenail concentrations of arsenic were also negatively associated with LINE-1 methylation. On the other hand, LINE-1 methylation levels were positively associated with toenail concentrations of iron and nickel, and with seven variants in *DNMT3A, TCN2, AS3MT, SLC19A1,* and *MTHFS* genes.

Our findings support previous results showing that females have significantly lower levels of LINE-1 methylation (El-Maarri et al. 2007; El-Maarri et al. 2011; Wilhelm et al. 2010; Zhu et al. 2012). DNA methylation is important for X-chromosome inactivation in females (Jones and Liang 2009), and although LINE-1 sequences do not seem to be the major mechanism involved in this process, they may be involved in spreading the X-inactivation signal across the chromosome (Bailey et al. 2000). In support of this, a recent study showed that LINE-1 sequences were hypomethylated in the inactive X-chromosome (Singer et al. 2012). A small study of 33 men and 33 women reported lower levels of blood SAM in women (Poirier et al. 2001). Hormonal factors may also contribute to the difference in methylation levels between genders. However, a recent *in vitro* study assessing the role of estrogen, progesterone and dihydrotestosterone on DNA methylation in four cell lines found no detectable effect of these

hormones on methylation levels at the LINE-1 and *Alu* repeats (El-Maarri et al. 2011). Further studies are needed to decipher the relationship between gender and LINE-1 methylation.

Because tobacco smoking is an important contributor to disease and is a modifiable behavioral factor, there has been much interest in the relationship between smoking and DNA methylation. Our findings are in line with other studies that reported no association between LINE-1 methylation and smoking status (Terry et al. 2011). In the present study, we found that subjects who smoked blond tobacco had lower levels of global DNA methylation than nonsmokers. An experimental study has shown that cigarette smoke condensates can induce DNA demethylation in repeat elements, such as LINE-1 and D4Z4 (Liu et al. 2010). Both black and blond tobacco cause disease although the former is more mutagenic, reflective of the higher levels of *N*-nitrosamines and aromatic amines in smoke produced by black tobacco (Malaveille et al. 1989). Our findings suggest that the toxic effects of blond tobacco could be mediated by modulating the epigenetic landscape. This may have a public health implication given epigenetic alterations are reversible.

We also provide evidence that arsenic levels were inversely associated with LINE-1 methylation, and that arsenic may have a strong effect on LINE-1 methylation. For each μg/g increase in arsenic there was a 3.6% decrease in DNA methylation level. This inverse association is in agreement with that from a population-based study that used a similar assay to assess LINE-1 methylation levels and toenail concentrations of arsenic (Wilhelm et al. 2010), as well as with several other experimental studies (Reichard and Puga 2010; Ren et al. 2011). The mechanisms through which arsenic exposure influences DNA methylation are not fully understood. Studies in cell lines and mouse models exposed to arsenic for up to 22 and 48 weeks, respectively have shown that prolonged exposure to sodium arsenite resulted in decreased global DNA

methylation, and inhibition of *DNMT1, DNMT3A,* and *DNMT3B* gene expression (Reichard and Puga 2010; Ren et al. 2011). It is likely that through the combined effect of depleting the cellular pool of SAM and inhibiting the activity of *DNMTs*, both inorganic and organic arsenic may lead to decreased global DNA methylation.

We are not aware of any human studies associating iron and nickel levels and global DNA methylation. In the present study, iron and nickel showed a small but significant positive association with LINE-1 methylation level. Genes involved in hepatocellular carcinoma (HCC) have been found to be hypermethylated in hereditary hemochromatosis, a disease characterized by chronic iron overload that is a risk factor for HCC (Lehmann et al. 2007). Iron, together with 2-oxoglutarate and oxygen, is an essential cofactor for the ten-eleven translocation (*TET*) family of proteins that hydroxylate 5-methylcytosine to 5-hydroxymethylcytosine and further oxidize to 5-carboxylcytosine and 5-formylcytosine, which have all been suggested to be precursors for both active and passive DNA demethylation (Bhutani et al. 2011). Experimental studies conducted in Chinese hamster cell lines (G12) treated with nickel chloride for up to 3 weeks have shown that nickel chloride leads to both promoter hypermethylation and elevated total genomic DNA methylation (Lee et al. 1995; Lee et al. 1998). How nickel induces DNA methylation is not yet understood, but it has been proposed that nickel first induces chromatin condensation followed by *de novo* methylation of heterochromatic DNA (Lee et al. 1995).

The three SNPs with the strongest associations with LINE-1 methylation were all in *TCN2*, including two exonic SNPs that result in missense substitutions (rs9606756 and rs9621049) and one intronic SNP (rs4820887). SNPs rs9621049 and rs4820887 have a linkage disequilibrium r^2 value of 0.8 implying that the observed effect in LINE-1 methylation may eventually be attributed to either of them. *TCN2* encodes for transcobalamin II which binds and transports

vitamin B12 into the cell (Regec et al. 1995), which suggests that variations in *TCN2* could potentially impair the one-carbon metabolism pathway by altering the cytoplasmic concentration of vitamin B12. *TCN2*-rs9606756 leads to an I23V substitution located at a NAGNAG tandem acceptor site that is a target of alternative splicing (Hiller et al. 2006). *TCN2*-rs9621049 leads to a S348F and may also play a role in the availability of vitamin B12 in the cell thereby affecting LINE-1 methylation levels.

Four SNPs in other genes (*DNMT3A*-rs7581217; *AS3MT*-rs7085104; *MTHFS*-rs1380642; *SLC19A1*-rs914238) involved in the one-carbon metabolism were also associated with global DNA methylation in our study population. *DNMT3A*, a *de novo* DNA methyltransferase, establishes the patterns of methylation in early embryonic development, along with *DNMT3B*, and cooperates with *DNMT1* to maintain the methylation of repetitive sequences, such as LINE-1 and *Alu* elements (Jones and Liang 2009). Recurrent mutations in *DNMT3A* have been associated with adult hematologic malignancies (Ley et al. 2010; Yan et al. 2011), and mice lacking *Dnmt3a* die in the first weeks of postnatal life (Robertson 2005). The product of *AS3MT* catalyzes the conversion of trivalent arsenic by addition of a methyl group to monomethylarsonic acid and dimethylarsonic acid (Ren et al. 2011); monomethylarsonic acid being the most toxic metabolite (Engstrom et al. 2011). rs7085104, located in the promoter region of *AS3MT*, has been associated with arsenic metabolism, as evidenced by differences in urinary concentration of arsenic metabolites (Engstrom et al. 2011; Valenzuela et al. 2009). We also observed a significant interaction of this SNP with levels of arsenic on LINE-1 methylation levels. While subjects with at least one copy of the major allele had a 4.1% decrease in methylation level for 1-μg/g increase in arsenic, which is comparable to the overall population (-3.6%), those homozygous for the variant allele had a 10% increase in LINE-1 methylation. These findings

support the putative functionality of the association. The product of *MTHFS* catalyzes the conversion of 5-formyltetrahydrofolate to 5,10-methenyltetrahydrofolate and a genome-wide association study reported an association between a variant in this gene and chronic kidney disease (Kottgen et al. 2008). *SLC19A1* is a ubiquitously expressed major transporter of folate and antifolates and regulator of the intracellular concentrations of folate (Matherly et al. 2007). Common variants in this gene have been associated with plasma folate levels, various types of cancer (esophageal, gastric and acute lymphoblastic leukemia), and altered methotrexate transport and adverse effects of methotrexate (Matherly et al. 2007).

Among the limitations of the study is the majority of individuals were of advanced age (mean age 64 years, SD=10 years) and men. This may explain the lack of association between DNA methylation and age in our study population, in contrast with other studies that included subjects with a broader age range (Fraga et al. 2005b). Thus, our findings refer to an adult population of mostly men. Results were consistent with estimates for the population as a whole when stratified by gender, with the exception of nickel, tobacco type, and *MTHFS*-rs1380642 which become nonsignificant while the point estimates were in the same direction (data not shown). These differences may reflect reduced power to estimate associations among women due to the small sample size. The presence of missing data for some of the variables might have resulted in decreased power but even with the available sample size we were able to reproduce previous results and identify novel predictors of LINE-1 methylation. Furthermore, while the study subjects were recruited from hospitals, none of reasons for hospitalization were significantly associated with LINE-1 methylation.

Strengths of the study include its size, the availability and quality of individual data on demographics, lifestyle, environmental exposures, and genetics. Additionally, we assessed

LINE-1 methylation levels, which are considered a good marker of global DNA methylation (Yang et al. 2004), using pyrosequencing, which gives accurate and reproducible measurements (Estecio et al. 2007; Laird 2010; Tost and Gut 2007). Furthermore, this assessment was made using DNA from granulocytes, avoiding a possible effect of cell blood count in our study.

To the best of our knowledge, this is the first study to identify seven SNPs in association to changes in LINE-1 methylation and to integrate different types of information to assess the determinants of global methylation in blood DNA. Integration of both internal and external exposure data in this study is a step forward in understanding how the exposome modulates DNA methylation patterns.

In conclusion, the current study provides further evidence that DNA methylation levels are influenced by variants in genes involved in the one-carbon metabolism pathway, and exposure to trace elements and tobacco smoke. Given the fact that smoking and some of the genetic variants and trace elements associated with LINE-1 methylation in the present study have also been associated with adverse health outcomes including cancer, our results provide additional insight into the potential mechanism through which these agents participate in the development of those diseases. Furthermore, these factors should be considered as potential confounders in etiologic and interventional studies analyzing the role of DNA methylation in disease. Nevertheless, future studies are required to replicate and extend our findings in different populations.

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Table 1. Association between LINE-1 methylation level and individual characteristics of study subjects in the SBC/EPICURO Study

Variables	N	LINE-1 methylation Mean (95% CI)	Unadjusted β (95% CI)	P-value	N	Adjusted β (95% CI)[a,b]	P-value
Age (years)	892	—	-0.004 (-0.02, 0.01)	0.6	889	-0.006 (-0.02, 0.01)	0.5
Gender							
Male	792	59.0 (58.6, 59.4)	Ref		789	Ref	
Female	100	58.0 (57.1, 58.8)	-0.4 (-0.9, 0.05)	0.08	100	-0.7 (-1.2, -0.1)	0.02
Region							
Barcelona	168	59.3 (58.5, 60.2)	Ref		168	Ref	
Vallès	135	58.3 (57.5, 59.0)	-0.2 (-0.7, 0.4)	0.5	135	-0.2 (-0.7, 0.3)	0.5
Elche	73	58.2 (57.1, 59.3)	-0.4 (-1.1, 0.2)	0.2	73	-0.5 (-1.1, 0.2)	0.1
Tenerife	145	58.6 (57.8, 59.4)	-0.1 (-0.7, 0.4)	0.7	144	-0.1 (-0.6, 0.4)	0.7
Asturias	371	59.2 (58.6, 59.8)	0.2 (-0.3, 0.6)	0.5	369	0.1 (-0.3, 0.6)	0.6
Body mass index (kg/m²)							
<25.0	372	58.8 (58.3, 59.3)	Ref		370	Ref	
25.0-26.99	148	58.9 (57.9, 59.9)	-0.2 (-0.6, 0.3)	0.5	148	-0.2 (-0.6, 0.3)	0.4
27.0-29.99	120	59.0 (58.1, 59.8)	0.1 (-0.3, 0.6)	0.6	120	0.2 (-0.3, 0.7)	0.4
≥30.0	57	58.8 (57.5, 60.2)	-0.08 (-0.7, 0.6)	0.8	57	-0.03 (-0.7, 0.6)	0.9
Missing data	195						
Smoking status							
Non-smoker	255	58.2 (57.7, 58.7)	Ref		255	Ref	
Occasional smoker	66	60.0 (58.4, 61.5)	0.4 (-0.2, 1.1)	0.2	66	0.3 (-0.3, 1.0)	0.3
Former smoker	329	58.9 (58.3, 59.5)	-0.2 (-0.5, 0.3)	0.6	329	-0.3 (-0.7, 0.1)	0.2
Current smoker	239	59.4 (58.6, 60.2)	-0.2 (-0.6, 0.2)	0.4	239	-0.4 (-0.9, 0.07)	0.1
Missing data	3						
Tobacco type							
Non-smoker	255	58.2 (57.7, 58.7)	Ref		255	Ref	
Blond only	99	58.5 (57.6, 59.5)	-0.5 (-1.0, 0.09)	0.1	99	-0.7 (-1.3, -0.08)	0.03
Black only	219	59.6 (58.8, 60.4)	0.06 (-0.4, 0.5)	0.8	218	-0.2 (-0.6, 0.3)	0.5
Blond and black	154	58.7 (57.8, 59.6)	-0.3 (-0.8, 0.2)	0.2	154	-0.6 (-1.1, -0.07)	0.03
Unknown	97	59.3 (58.2, 60.5)	-0.01 (-0.6, 0.6)	0.9	97	-0.12 (-0.7, 0.5)	0.7

Variables	N	LINE-1 methylation Mean (95% CI)	Unadjusted β (95% CI)	P-value	N	Adjusted β (95% CI)[a,b]	P-value
Missing data	68						
Controls' diagnosis							
Hernia	332	58.8 (58.2, 59.4)	Ref		330	Ref	
Fracture & Trauma	263	59.3 (58.6, 60.0)	-0.2 (-0.5, 0.2)	0.4	262	-0.02 (-0.4, 0.4)	0.9
Hydrocele	122	58.7 (57.8, 59.6)	0.2 (-0.3, 0.7)	0.4	122	0.2 (-0.3, 0.7)	0.5
Other Abdominal Surgery	99	58.3 (57.4, 59.2)	-0.2 (-0.7, 0.3)	0.4	99	-0.09 (-0.6, 0.5)	0.7
Other Diseases	76	59.2 (57.9, 60.5)	0.01 (-0.9, 0.6)	0.9	76	0.2 (-0.4, 0.8)	0.5
Dietary intake[c]							
Vitamin B1 (µg/day/kcal)	645	–	0.5 (-0.6, 1.6)	0.3	644	0.6 (-0.6, 1.7)	0.3
Vitamin B2 (µg/day/kcal)	645	–	0.1 (-0.5, 0.8)	0.7	644	0.2 (-0.5, 0.8)	0.6
Vitamin B3 (µg/day/kcal)	645	–	0.01 (-0.05, 0.08)	0.7	644	0.02 (-0.05, 0.09)	0.5
Vitamin B6 (µg/day/kcal)	645	–	0.6 (-0.2, 1.4)	0.1	644	0.8 (-0.05, 1.6)	0.07
Vitamin B12 (µg/day/kcal)	645	–	-0.04 (-0.09, 0.01)	0.1	644	-0.03 (-0.08, 0.02)	0.3
Folate (µg/day/kcal)	645	–	0.001 (-0.002, 0.004)	0.4	644	0.003 (-0.001, 0.01)	0.1
Protein (µg/day/kcal)	645	–	0.01 (-0.01, 0.03)	0.4	644	0.01 (-0.01, 0.03)	0.4
Alcohol (µg/day/kcal)	645	–	-0.002 (-0.02, 0.02)	0.8	644	-0.01 (-0.03, 0.02)	0.5
Fruit (g/day/kcal)	639	–	0.0001 (-0.001, 0.002)	0.9	638	0.0001 (-0.001, 0.002)	0.9
Vegetable (g/day/kcal)	640	–	0.001 (-0.001, 0.003)	0.3	639	0.002 (-0.001, 0.004)	0.1
Fruit and vegetable (g/day/kcal)	639	–	0.0003 (-0.0008, 0.001)	0.6	638	0.0005 (-0.0007, 0.002)	0.4
Toenail trace elements[d]							
Aluminum (µg/g)	658	–	-0.003 (-0.008, 0.002)	0.2	658	-0.003 (-0.008, 0.002)	0.2
Arsenic (µg/g)	659	–	-2.9 (-5.2, -0.6)	0.02	659	-3.6 (-5.9, -1.2)	0.003
Cadmium (µg/g)	659	–	0.08 (-0.4, 0.5)	0.7	659	0.1 (-0.3, 0.6)	0.6
Chromium (µg/g)	658	–	0.06 (-0.01, 0.1)	0.09	659	-0.01 (-0.05, 0.03)	0.6
Copper (µg/g)	659	–	-0.002 (-0.06, 0.05)	0.95	659	-0.01 (-0.07, 0.04)	0.6
Iron (µg/g)	657	–	-0.002 (-0.006, 0.002)	0.4	658	0.002 (0.001, 0.004)	0.009
Lead (µg/g)	659	–	-0.05 (-0.1, 0.03)	0.2	659	-0.06 (-0.1, 0.02)	0.2
Manganese (µg/g)	659	–	-0.03 (-0.1, 0.09)	0.7	659	-0.05 (-0.2, 0.06)	0.4
Nickel (µg/g)	659	–	0.02 (0.006, 0.03)	0.002	659	0.02 (0.005, 0.03)	0.004
Selenium (µg/g)	659	–	0.1 (-0.8, 1.0)	0.8	659	0.2 (-0.7 1.2)	0.6
Vanadium (µg/g)	651	–	-0.7 (-2.7, 1.3)	0.5	651	-0.9 (-2.8, 1.2)	0.4

Variables	N	LINE-1 methylation Mean (95% CI)	Unadjusted β (95% CI)	P-value	N	Adjusted β (95% CI)[a,b]	P-value
Zinc (μg/g)	659	—	-0.002 (-0.004, 0.001)	0.2	659	-0.001 (-0.004, 0.002)	0.4
***NAT2* phenotype**							
Rapid/Intermediate acetylator	389	59.0 (58.4, 59.6)	Ref		388	Ref	
Slow acetylator	498	58.8 (58.4, 59.2)	0.2 (-0.1, 0.5)	0.3	496	0.2 (-0.1, 0.5)	0.2
Missing data	5						
***GSTM1* genotype**							
(+/+, +/-)	421	58.9 (58.3, 59.4)	Ref		419	Ref	
(-/-)	462	59.0 (58.5, 59.4)	0.04 (-0.3, 0.4)	0.8	461	0.01 (-0.3, 0.3)	0.9
Missing data	9						
***GSTT1* genotype**							
(+/+, +/-)	688	59.0 (58.6, 59.4)	Ref		685	Ref	
(-/-)	198	58.5 (57.9, 59.2)	-0.2 (-0.6, 0.2)	0.3	198	-0.2 (-0.6, 0.2)	0.4
Missing data	6						

[a]Adjusted for age, gender, region, and smoking status (non-, occasional, former, current smoker). Tobacco type's β is not adjusted for smoking status.

[b]The number of observations are reduced by three because of missing data on smoking status.

[c]Data available for those who completed food frequency questionnaire.

[d]Data available for those who provided toenail for trace element assessment.

Note: the exposure contrast for trace elements is 1-μg/g and for dietary variables is 1-μg/day/kcal.

Table 2. Association between LINE-1 methylation levels and single nucleotide polymorphisms in genes involved in the one-carbon metabolism pathway

Gene	dbSNP [Chromosome, position in the gene, location[a]]	MAF	N	MOI	Genotype	Unadjusted β (95% CI)	P-value	Adjusted β[b] (95% CI)	P-value
DNMT3A	rs7581217 [2, intron, 25378448]	0.39	875	Additive	per allele T	0.3 (0.1, 0.6)	0.003	0.3 (0.1, 0.6)	0.002
AS3MT	rs7085104 [10, flanking 5'UTR, 104618863]	0.38	751	Recessive	AA/AG	Ref		Ref	
			124		GG	0.8 (0.3, 1.2)	0.0008	0.7 (0.3, 1.2)	0.001
MTHFS[c]	rs1380642 [15, flanking 3'UTR, 77883926]	0.18	585	Codominant	CC	Ref	0.03	Ref	0.05
			258		CT	0.3 (-0.05, 0.6)		0.3 (-0.08, 0.6)	
			32		TT	-0.8 (-1.6, 0.07)		-0.8 (-1.6, 0.09)	
SLC19A1[c]	rs914238 [21, flanking 5'UTR, 45840089]	0.49	231	Codominant	TT	Ref	0.0008	Ref	0.0007
			435		TC	0.5 (0.09, 0.8)		0.5 (0.08, 0.8)	
			209		CC	-0.2 (-0.7, 0.2)		-0.3 (-0.7, 0.2)	
TCN2[d]	rs9621049 [22, exon, 29343419]	0.11	864	Recessive	CC/CT	Ref		Ref	
			11		TT	4.5 (3.1, 5.9)	4.3×10^{-10}	4.2 (2.8, 5.7)	4.7×10^{-9}
	rs9606756 [22, exon, 29336860]	0.12	864	Recessive	AA/AG	Ref		Ref	
			11		GG	2.2 (0.8, 3.6)	0.003	1.9 (0.5, 3.3)	0.008
	rs4820887 [22, intron, 29346914]	0.10	866	Recessive	GG/GA	Ref		Ref	
			9		AA	4.6 (3.0, 6.2)	9.3×10^{-9}	4.0 (2.5, 5.6)	4.8×10^{-7}

MAF, minor allele frequency; MOI, mode of inheritance.
[a]Human Genome Build 36.3 location.
[b]Adjusted for age, gender, region, and smoking status.
[c]Global p-value for rs1380642 and rs914238 was estimated by using a two-degrees of freedom likelihood-ratio test.
[d]Linkage disequilibrium (rs4820887 vs. rs9621049) $r^2 = 0.8$.